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A COMPARATIVE STUDY OF CHROMOSOMAL POLYMORPHISM IN SIBLING SPECIES OF THE *WILLISTONI* GROUP OF DROSOPHILA[1]

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INTRODUCTION

NATURAL populations of many species of Drosophila are polymorphic with respect to the gene arrangement in their chromosomes. At least in some species, this polymorphism, caused by inversions of chromosome segments, is adaptive and it is balanced. Inversion heterozygotes are superior in adaptive values to either of the corresponding homozygotes. Superiority of the heterozygotes leads, in a sexual cross-fertilizing species, to establishment of equilibria at which all the different gene arrangements present in a population occur with definite frequencies. Furthermore, the adaptive values of the chromosomal types may vary in different environments to which the species is exposed in space or in time. In general, the more polymorphic a species is, the more environments it can use or control. Polymorphism is a device to cope with the diversity of environments (Dobzhansky, 1947, 1949).

The amount of chromosomal polymorphism present in populations of different species, and in populations of the same species in different parts of its geographic distribu-

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tion, is often quite variable. Warters (1944) found no inversions at all in populations of *Drosophila hydei*, while at least 40 different inversions are known in *D. willistoni*. In the state of Goyaz, in central Brazil, a female of *D. willistoni* is heterozygous, on the average, for 9.4 ± 0.3 inversions, while on the margin of the "caatinga" desert of Bahia, Brazil, only 0.81 ± 0.03 inversions per individual are found. The variations in the concentration of inversions in different bioclimatic regions of Brazil are thus very considerable, but they are comprehensible in the light of the hypothesis that the amount of adaptive polymorphism in a population is proportional to the variety of environments (ecological niches) which the population occupies. Since *D. willistoni* lives mainly on fermenting fruits, the equatorial rainforests and savannas of central and northern Brazil offer a much greater diversity of environments than do the deserts in which the species reaches its toleration limit. It should also be kept in mind that, other things being equal, a species is more likely to have become adapted to diverse environments where it has lived for a long time than at the margins of its distribution area where it is a relative newcomer. Finally, the presence of several competing species with kindred ecological requirements in the same territory restricts each of them to fewer habitats than they would occupy in the absence of competitors (da Cunha, Burla, Dobzhansky, 1950).

The present article reports an attempt to test the validity of the above hypothesis by making a comparison of the chromosomal polymorphism in a group of four morphologically as well as ecologically closely related species. These are the four sibling species of the *willistoni* group, namely, *D. willistoni* Sturtevant, *D. paulistorum* Dobzhansky and Pavan, *D. tropicalis* Burla and da Cunha, and *D. equinoxialis* Dobzhansky. Some of them have a much greater chromosomal polymorphism than others, and some are common and widespread, while others are relatively rare and restricted to smaller geographic areas.

Distribution and Frequency of the Sibling Species

The four sibling species of the *willistoni* group are morphologically so nearly identical that the safest way to distinguish them is to examine their salivary gland chromosomes or to make crosses to known strains of the respective species (Burla *et al.*, 1949). Although the geographic distribution areas of the species are by no means completely known, some facts important for our purposes are established.

D. willistoni has a much wider distribution than the others. It has been found by Patterson and Wagner (1943) in southernmost Florida, and we have ascertained that the strains collected for us by Professor J. A. Moore in Axtla, San Luis Potosi, Mexico, belong to the same species. The southernmost known findings are Uruguaiana, Santo Angelo and Reuter, state of Rio Grande do Sul, Brazil (the Uruguaiana and Reuter collections by Professor A. R. Cordeiro). *D. paulistorum* extends from Costa Rica and Trinidad to the coasts of São Paulo and Paraná in Brazil; its distribution is, therefore, included in that of *D. willistoni.* The area of *D. tropicalis* is included in that of *D. paulistorum;* the former species extends from the territory of Rio Branco to central Goyaz in Brazil and Santa Cruz de la Sierra, Bolivia, where it has been collected by Dr. W. Kerr. Finally, *D. equinoxialis* occurs only in the Amazonian rainforests (Table 1).

The distribution areas of the four species thus decrease in the order *willistoni* > *paulistorum* > *tropicalis* > *equinoxialis,* the smaller areas being always included in the larger, so that in the center all four species are sympatric. This fact is in itself presumptive evidence that the species with larger areas are ecologically more versatile than those with smaller ones. Confirmatory evidence comes from observations on the habitats in which the species occur. *D. willistoni* has been found in every one of the 17 bio-

TABLE I

Relative Abundance (in per cent.) of the Four Sibling Species of the *Willistoni* Group

Region	*willistoni*	*paulistorum*	*tropicalis*	*equinoxialis*	Flies studied
Costa Rica	63	37	...	...	16
Trinidad	67	33	...	...	3
Equatorial Brazil					
Rio Branco, Savanna	74	25	1	1	110
Rio Branco, Mucajaí	15	60	...	25	73
Rio Negro	49	43	3	5	154
Marajó Island	69	4	27	...	49
Belem, Para	26	60	6	8	519
Acre and Guaporé.	47	33	12	8	372
Central Brazil and Bolivia					
Imperatriz, Maranhão	96	3	1	...	104
Carolina, Maranhão	87	11	3	...	37
Catuní, Bahia	100	...	...	...	150
Palma, Goyaz	95	1	4	...	76
Monjolinho, Goyaz	89	11	...	...	144
Santa Cruz, Bolivia	76	18	6	...	13
Southern Brazil					
Pirassununga, São Paulo	95	5	...	...	581
Mogí, São Paulo ..	67	33	...	...	354
Vila Atlantica, São Paulo	31	69	...	...	393
Paranaguá, Paraná	83	17	...	...	29
Iguassú, Paraná ..	100	...	...	...	134
Reuter, Rio Grande Sul	100	...	...	...	27
Santo Angelo, Rio Grande Sul	100	...	...	...	27

climatic regions and 35 localities in Brazil in which collecting has been attempted (Dobzhansky and Pavan, 1950). Its habitats range from the superhumid forests on the coast of southern Brazil and on the Rio Negro close to the Equator, to the caatinga desert in the state of Bahia. *D. paulistorum* occurs mainly in the rainforests and avoids the drier savannas. In the state of São Paulo, *D. paulistorum* is

much more abundant on the wet coast than in the interior, and more abundant during the hot and wet season than during the cool and dry season. It is, however, more common than *D. willistoni* apparently at all seasons in the equatorial forest near Belem, Pará. *D. tropicalis* and *D. equinoxialis* are seldom common anywhere; exceptionally high frequencies of the former have been encountered only in the savannas of Rio Branco, and of the latter in the swampland forests at Cape Maguarí, Marajó Island. On the assumption that the chromosomal polymorphism and the ecological versatility are correlated, the amounts of the former are expected to decrease in the order *willistoni* > *paulistorum* > *tropicalis* > *equinoxialis*.

Total Numbers of Inversions in the Four Species

All known species of the *willistoni* group, numbering nine, have the same metaphasic chromosomes: metacentric X and metacentric second chromosomes, and acrocentric third chromosomes. Five chromosome limbs, XL, XR, II L, II R, and III, occur in the salivary gland cells. The disc patterns are sufficiently similar in the homologous chromosomes of the four sibling species under consideration, so that the chromosomes can be easily identified (except the third, which is rather more differentiated in the different species, see Burla *et al.*, 1949, and Dobzhansky, 1950). The chromosomes of *D. willistoni* have been studied in most detail, since more than 2,500 females from various parts of Brazil have been tested by examination of the gene arrangements in the larval salivary gland cells in the F_1 offspring of the flies collected in their natural habitats (da Cunha *et al.*, 1950). Here we need only a summary of the numbers of different inversions found in the chromosomes of *D. willistoni:*

XL	9
XR	5
II L	8
II R	5
III	13
Total	40

Some 800 females of *D. paulistorum* were tested by examination of the salivary gland chromosomes in their F_1 progeny. The corresponding figures for *D. tropicalis* and *D. equinoxialis* are 74 and 79. In all cases, wild females, inseminated in nature by one or more males, were placed singly in culture bottles; when mature larvae appeared in the cultures, acetic orceine smears of the salivary glands were made and examined while fresh; the gene arrangements were recorded in a single larva from each culture. If the larva happened to be a male only heterozygous inversions in the autosomes could be scored, while in female larvae the autosomes as well as the X-chromosomes were studied. The numbers of the autosomes are, therefore, larger than of the X-chromosomes studied.

The populations of *D. paulistorum* proved to contain a great variety of inversions, some of them found only in one or a few localities. This made the study difficult; the interrelationships of some multiple inversions, especially in the third chromosome, could not be unraveled. The figures given below are minimum estimates of the numbers of kinds of inversions found in *D. paulistorum:*

XL	6
XR	3
II L	5
II R	5
III	15
Total	34

The rare species, *D. tropicalis* and *D. equinoxialis,* have much fewer inversions than *D. willistoni* and *D. paulistorum,* and the differences are clearly significant despite the limited material at our disposal for the former two species. The observed figures are as follows:

D. tropicalis	
II L	2
III	2
Total	4

D. equinoxialis	
XR	1
II R	1
III	2
Total	4

Description of Inversion in *D. paulistorum*

The inversions in *D. willistoni* have been described by da Cunha *et al.* (1950). The following descriptions of the inversions in *D. paulistorum* are made exclusively for purposes of identification. An analysis of their phylogenetic relationships would require systematic crosses of lines of different geographic origin to a standard strain, and this has not yet been done. In the following descriptions, the inversions in each chromosome limb are designated by letters, beginning with the proximal (closest to the chromocenter), and down to the distal (free) end of the chromosome.

The left limb of the X chromosome (XL):

Inversion A: A fairly long inversion located proximally from the middle of the length of the chromosome, as shown in Fig. 7; found at Belem, along the Rio Negro, Rio Branco, and in Acre.

Inversion B: An inversion located somewhat distally from Inversion A, probably overlapping; shown in Fig. 6 in the middle; found on Rio Negro, Rio Branco, and in Acre.

Inversion C: Includes the middle of the chromosome, beginning just proximally to a light "bulb" present in this part of the chromosome in south Brazilian strains. This is the proximal one of the two inversions shown in XL in Plate 1 in the paper of Burla *et al.*, 1949 (p. 304). Common in southern Brazil, encountered also in Goyaz and in Bolivia.

Inversion D: A fairly long inversion just distal from C and apparently not overlapping the latter. This is the distal of the two inversions in the drawing just cited. Encountered only on the coast of São Paulo and in Bolivia.

Inversion E: A rather short subterminal inversion shown in Fig. 5. Recorded for Goyaz, Acre, and Rio Branco.

Inversion F: A very short subterminal inversion found in a single strain from Cruzeiro do Sul, Acre, and shown in Fig. 5.

The right limb of the X chromosome (XR):

Inversion A: A long inversion, including most of the proximal half of the chromosome, shown in Fig. 11; found near Belem, in Acre, and in Rio Mucajai.

Inversion B: An inversion much shorter than Inversion A and having the distal break at almost the same point as Inversion A, consequently included in Inversion A. Occurs in the state of São Paulo.

Inversion C: An inversion of about the same length as Inversion A, but lying more distally; the distal break just distal from the "repeat area"

shown in Fig. 11 as undifferentiated dark gray "bulb"; found in a single strain from Goyaz.

The left limb of the second chromosome (II L):

Inversion A: Includes the distal three-fifths of the length of the chromosome, having the distal break in the terminal position, or very nearly the terminal position. Shown in Plate 2 of da Cunha *et al.* (1950). Fairly common in the state of São Paulo.

Inversion B: A median inversion which includes about a quarter of the total length of the chromosome; included in Inversion A; occurs in the basin of the Amazon.

Inversion C: A subterminal inversion which is included in Inversion A and overlaps Inversion B; found in a single strain from Costa Rica. Apart from these, two other inversions have been found, one in a strain from Rio Negro and one from Belem, which have not been sufficiently studied.

The right limb of the second chromosome (II R):

Inversion A: Includes about half of the length of the chromosome, beginning some 50 discs from the chromocenter, and extending to just beyond the dark heterochromatic "repeat area" in the middle; encountered in a single strain from Mucajaí, territory of Rio Branco.

Inversion B: Overlaps Inversion A slightly, since the proximal end lies distal to the heterochromatic area mentioned in the description of Inversion A; a drawing of Inversion B is in Plate 2, da Cunha *et al.* (1950). Recorded from Belem, Rio Negro, Rio Branco, and Acre. A fly from Rio Negro was heterozygous for both Inversion A and Inversion B, the compound giving a typical configuration of two overlapping inversions, the heterochromatic area being included in the zone of the overlap.

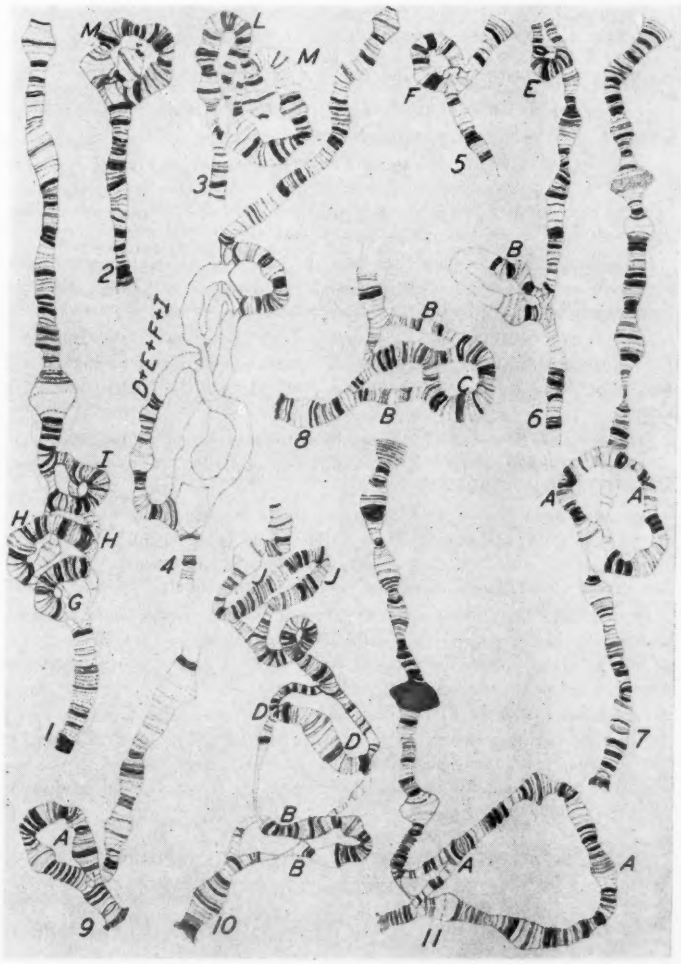
Inversion C: A moderately short subterminal inversion, the proximal break being located distal to the heterochromatic area, not overlapping either Inversion A or Inversion B; Rio Mucajaí, in the territory of Rio Branco.

Inversion D: An apparently terminal inversion, overlapping Inversion C. This inversion was seen alone in a chromosome from Rio Negro, and together with Inversion C, as a double overlapping inversion, in chromosomes from Rio Negro and from Rio Mucajaí. Another subterminal inversion, not identical with Inversion C, occurred in a strain from Belem, but has not been drawn or studied sufficiently to make a description.

The third chromosome (III):

This chromosome contains the greatest diversity of inversions in the species under consideration.

Inversion A: A fairly short subbasal inversion shown in Fig. 9. Occurs in the state of São Paulo, but has been recorded from Goyaz as well; this latter record may possibly refer to a distinct inversion in the same part of the chromosome.



FIGS. 1–11, some of the inversions found in the third chromosome (Figs. 1–4, 8–10), in the left limb of the X-chromosome (Figs. 5–7), and in the right limb of the X-chromosome (Fig. 11) of *Drosophila paulistorum*. The drawings have been made with the aid of a camera lucida, usually only a part of the total length of the chromosome containing inversions being represented; where some sections of chromosomes could not be seen clearly they are represented by dotted outlines, as in Figs. 4 and 6.

Inversion B: A subbasal inversion somewhat longer than Inversion A and beginning slightly more distally; shown in Fig. 8 and Fig. 10; recorded from Acre, Guaporé, Rio Negro, and Belem.

Inversion C: A short inversion included in Inversion B and shown in Fig. 8 together with the latter; found in São Paulo, and also in Acre, Guaporé, Bolivia, Goyaz, and Rio Negro.

Inversion D: A fairly long median inversion shown in Fig. 10, and also in Plate 3 of the paper by da Cunha *et al.* (1950). This inversion, or inversions very similar to each other in this part of the chromosome, occur in São Paulo, Goyaz, Maranhão, Belem, and Acre.

Inversion E: An inversion just distal to Inversion D and apparently independent of the latter, shown in Plate 3, da Cunha *et al.* (1950); recorded from Belem, Rio Negro, Rio Mucajaí, and Acre.

Inversion F: An inversion overlapping both Inversion D and Inversion E, and giving within them a complex configuration shown in Fig. 4 and encountered in several chromosomes from Acre, Rio Negro, and Rio Branco.

Inversions G, H, and I: Three apparently non-overlapping tandem inversions shown in Fig. 1 and encountered, always together, in several chromosomes from São Paulo.

Inversion J: A fairly long inversion in the distal part of the chromosome, shown in Fig. 10, and also in Plate 3, da Cunha *et al.* (1950); occurs from Acre to Belem, and from Goyaz and Maranhão to Rio Branco. A chromosome from Costa Rica also seemed to contain this inversion.

Inversion K: An inversion in the same part of the chromosome as Inversion J, but clearly not identical with the latter; shown in Plate 3, da Cunha *et al.* (1950); occurs in the state of São Paulo. A single record for Goyaz probably refers to Inversion J.

Inversions L and M: Two short subterminal inversions shown in Fig. 2 and Fig. 3; occur at Belem, in Acre, along the Rio Negro and Rio Branco. Two further inversions have been seen, each in a single strain, in the offspring of flies collected along the Rio Negro, but they have not been sufficiently studied.

Geographic Distribution of Inversions in *D. paulistorum*

It has been shown in the description of the inversions that each of the latter occurs in a certain geographic region, and that the distribution regions of the different inversions often do not coincide. This can be seen even more clearly in Table 2 which summarizes the data on the incidence of the more frequently occurring inversions in different collections.

Only the inversions III D and possibly III C occur in all

biogeographic regions of Brazil in which the species has been collected in not too small numbers. Several inversions seem to be confined to, or at least occur frequently, in the Amazon basin (XL–A, II L–B, II R–B, III–E, III–J, III–L, III–M). Others occur only or mainly in southern and central Brazil (XL–C, II L–A, III–K). Still others,

TABLE 2

Frequencies (in per cent.) of the Principal Inversions Found in Heterozygous Condition in Populations of *D. paulistorum* from Different Geographic Regions

Chromosome and Inversion	Rio Branco, Savanna	Rio Branco, Mucajaí	Rio Negro	Belem, Pará	Acre and Guaporé	Monjolinho, Goyaz	Pirassununga, São Paulo	Mogí, São Paulo	Vila Atlantica, São Paulo
XL–A	5.0	11.2	3.2	15.7	5.2	...	...	...	...
XL–C	5.0	...	...	1.4	1.7	23.0	47.1	39.5	39.2
XL–E	...	...	...	...	1.7	46.1	...	...	...
XR–A	...	2.8	...	...	5.2	...	...	...	...
XR–B	...	...	...	...	...	...	17.7	1.1	1.2
II L–A	...	...	...	...	...	...	13.0	8.6	27.8
II L–B	11.2	37.5	11.4	10.5	4.7	...	...	...	...
II R–B	11.2	12.5	9.1	20.0	14.3	...	...	...	...
III–A	...	...	...	...	2.4	18.7	17.4	6.9	13.2
III–B	3.7	...	...	1.7	10.7	12.5	...	...	...
III–C	3.7	...	22.7	...	15.5	12.5	26.1	3.4	1.3
III–D	...	8.3	11.4	11.3	17.8	12.5	13.0	12.1	4.4
III–E	7.4	4.2	11.4	8.7	22.6	...	...	...	...
III–G +H +I..	...	...	...	...	...	...	...	6.1	10.1
III–J	3.7	...	15.9	15.6	10.7	12.5	...	...	...
III–K	...	...	...	...	...	68.7	21.7	6.1	8.9
III–L	3.7	2.1	6.8	6.1	9.4	...	...	...	...
III–M	...	...	2.3	3.5	10.7	...	...	...	...
Number of flies tested	27	48	44	115	84	16	23	116	316

not included in Table 2, have been found in a single region. This relatively narrow localization of the inversions in *D. paulistorum* contrasts with the situation in *D. willistoni*, in which many inversions occur in populations of all parts of Brazil (da Cunha *et al.*, 1950). It is tempting to correlate this difference with the greater ecological versatility of *D. willistoni* and the relatively greater specialization of

D. paulistorum. The distribution of *D. willistoni* is, in most of Brazil, probably more continuous than that of *D. paulistorum.*

Inversions in *D. tropicalis* and *D. equinoxialis*

Burla *et al.* (1950) have shown in Plate 1 an inversion in the subterminal portion of the third chromosome in *D. tropicalis.* It has been found in strains from Cruzeiro do Sul and Palmares, Acre, and in Rio Negro. One chromosome from Porto Velho, Guaporé, had this and also another more proximally located inversion in the third chromosome. Some II L chromosomes from Palmares, Porto Velho, and Santa Cruz in Bolivia respectively had double inversion in the middle third of the chromosome length. Most strains of *D. tropicalis* which we have studied were free of inversions.

In *D. equinoxialis,* an individual from Belem and one from Rio Negro carried a long inversion in the XR chromosome shown in Plate 1 (Burla *et al.,* 1949). It resembles inversion XR–A in *D. paulistorum,* but a careful comparison discloses perceptible differences. One individual from Belem and another from Cruzeiro do Sul, Acre, were heterozygous for inversions in the middle of the third chromosome. An individual from Belem had a subbasal inversion in II R, and one from Rio Negro had a median inversion in XL-chromosomes. All other flies of this species were homozygous for the gene arrangement.

Comparison of the Frequencies of Inversion Heterozygotes in This Sibling Species

We have recorded 40, 34, 4, and 4 inversions respectively in *D. willistoni, D. paulistorum, D. tropicalis,* and *D. equinoxialis.* Although more extensive studies would almost certainly discover further inversions in every one of these species, there can be no doubt at least that the widespread and ecologically versatile *D. willistoni* and *D. paulistorum* contain a greater diversity of gene arrangements than the

geographically and ecologically more limited *D. tropicalis* and *D. equinoxialis*. Even more significant than comparisons of the total numbers of kinds of inversions in species as wholes are mean numbers of inversions present in heterozygous condition in different species and populations. The incidence of inversion heterozygotes in natural populations

TABLE 3

Mean Numbers of Heterozygous Inversions per Individual of *D. willistoni* and *D. paulistorum* in Populations of Different Geographic Regions

Region	*Drosophila willistoni*		*Drosophila paulistorum*	
	Mean	Limits	Mean	Limits
Costa Rica	♀ 3.25 ± 0.85	2–4	♀ 1.67	1–3
	♂ 2.00 ± 0.71	1–3	?	?
Rio Branco, Savanna	♀ 4.77 ± 0.43	0–11	♀ 0.55 ± 0.20	0–2
	♂ 3.00 ± 0.23	0–7	♂ 0.29	0–2
Rio Branco, Mucajaí	♀ 2.67 ± 0.46	0–8	♀ 1.02 ± 0.16	0–4
	♂ 2.50 ± 0.49	0–5	♂ 0.92 ± 0.31	0–4
Rio Negro, Amazonas	♀ 5.03 ± 0.49	1–9	♀ 1.77 ± 0.31	0–6
	♂ 4.03 ± 0.36	0–7	♂ 1.38 ± 0.36	0–4
Belem, Pará	♀ 2.85 ± 0.18	0–7	♀ 1.13 ± 0.13	0–4
	♂ 2.53 ± 0.23	0–7	♂ 0.87 ± 0.14	0–3
Acre and Guaporé	♀ 5.10 ± 0.20	0–10	♀ 1.74 ± 0.19	0–7
	♂ 4.77 ± 0.26	0–9	♂ 1.77 ± 0.37	0–6
Monjolinho, Goyaz	♀ 9.36 ± 0.26	2–14	♀ 1.46 ± 0.26	0–3
	♂ 6.56 ± 0.31	2–10	♂ 1.67	0–3
Pirassununga, São Paulo	♀ 4.13 ± 0.10	0–11	♀ 1.45 ± 0.24	0–3
	♂ 3.52 ± 0.13	0–8	♂ 1.33	1–2
Mogí, São Paulo	♀ 3.97 ± 0.13	0–13	♀ 1.01 ± 0.12	0–4
	♂ 2.92 ± 0.34	0–9	♂ 0.56 ± 0.16	0–3
Vila Atlantica, São Paulo	♀ 3.06 ± 0.12	0–9	♀ 1.26 ± 0.08	0–6
	♂ 2.34 ± 0.12	0–7	♂ 0.77 ± 0.12	0–5

is the best measure we can devise of the amount of adaptive polymorphism which these populations have evolved in the process of adjustment to their environment. Indeed, a species as a whole may have many gene arrangements, but if it is geographically differentiated the local populations can be rather uniform; a species with more limited geographic

differentiation may be more diversified intra-populationally. We have seen indications that *D. willistoni* is geographically less differentiated than *D. paulistorum,* but the data in Table 3 leave no doubt that the populations of the former species are more polymorphic than those of the latter.

Without a single exception, populations of *D. paulistorum* have fewer heterozygous inversions per individual than sympatric populations of *D. willistoni* (da Cunha *et al.,* 1950, have shown, however, that some populations of the latter species, such as that of the caatinga desert in the state of Bahia, have fewer heterozygous inversions than certain populations of *D. paulistorum;* we have not secured a sample of *D. paulistorum* in the caatinga). The frequencies of heterozygous inversions in different populations of *D. paulistorum* are rather more uniform than in *D. willistoni* and, because of the smallness of our material from most geographic regions, significant differences are not numerous. In general, the populations of the interior part of the Amazon Valley (Rio Negro, Acre) seem to have more inversions than the other populations, but more extensive data would be needed to confirm this. There is no indication of a negative correlation between the frequencies of inversions in the populations of *D. willistoni* and *D. paulistorum,* but again the material is insufficient to say that such a relationship, which could be expected on theoretical grounds, does not exist.

The average numbers of inversions in *D. tropicalis* and *D. equinoxialis* are decidedly lower than in the other species, namely:

D. tropicalis	0.14 ± 0.06
D. equinoxialis (♀)	0.11 ± 0.05

Discussion

The hypothesis which we desire to test by our observations on the chromosomal inversions in the sibling species of the *willistoni* group is that the adaptive polymorphism

present in populations of a species is positively correlated with the diversity of environments which these populations exploit. This hypothesis is deduced from the principle, developed especially by Gause (1934) and by Lack (1947), according to which forms with identical ecological requirements can not live indefinitely in the same territory, because the more efficient form will eventually crowd out and supplant the less efficient ones. That the chromosomal polymorphism in Drosophila is adaptive has been proved by observations and experiments on several species: *D. funebris* (Dubinin and Tiniakov, 1946), *D. pseudoobscura* (Dobzhansky, 1947, 1949), *D. persimilis* (Spiess, 1950), and *D. robusta* (Carson and Stalker, 1949; Levitan, 1950). There is indirect evidence of adaptive value of some of the chromosome inversions in *D. willistoni* (da Cunha *et al.*, 1950). We assume that the chromosomal polymorphism is, in general, adaptive also in other Drosophila species. Granting that assumption, the data presented above confirm the value of the hypothesis. *D. willistoni* is ecologically more versatile than *D. paulistorum,* and the latter more than *D. tropicalis* and *D. equinoxialis* (Dobzhansky and Pavan, 1950). The numbers of inversions encountered in these species and the numbers of heterozygous inversions per individual in their populations are highest in *D. willistoni* and lowest in *D. tropicalis* and *D. equinoxialis.*

Sibling species, or otherwise closely related species, are most favorable materials for testing the hypothesis because their genetic and ecologic adaptive systems are most nearly comparable. It would be obviously invalid to compare the chromosomal polymorphism in Drosophila with that in grasshoppers, for example, because inversions are more easily established in forms in which no chiasmata occur in male meiosis than in those in which they do. It is nevertheless tempting to inquire to what extent the abundance of intra-populational inversions is correlated with ecological versatility in the genus *Drosophila* as a whole, or at least in the subgenus *Sophophora* to which the *willistoni* group

belongs. Although the amount of available information relevant to the problem is rather small, it seems that it is in fair accord with the hypothesis.

Three closely related species of the *obscura* group of *Sophophora* occur sympatrically in the Sierra Nevada of California. By and large, *D. pseudoobscura* is the most widespread, *D. persimilis* more limited, and *D. miranda* the most specialized species. The frequency of inversion heterozygotes is highest in *D. pseudoobscura* and lowest in *D. miranda* (Dobzhansky, 1948, and unpublished data). Apart from the *willistoni* group, one of the most widespread species in Brazil is *D. nebulosa,* which holds the first place in abundance in dry savanna and desert-like regions. The number of heterozygous inversions per individual of this species is about as great as in *D. paulistorum* (unpublished data of Professor A. Dreyfus and the present authors). On the other hand, preliminary examinations of some less common and ecologically limited Brazilian species, such as *D. bandeirantorum, D. dreyfusii, D. camargoi* (data of the authors and of Miss M. Wedel), and of *D. pallidipennis* (data of Dr. N. Freire Maia) show few or even no heterozygous inversions. Further observations bearing on this problem are needed.

Summary

Drosophila willistoni is the commonest and most widespread of the four sibling species of the *willistoni* group in Brazil; *D. paulistorum* is less, and *D. tropicalis* and *D. equinoxialis* are least common and widespread. In *D. willistoni* 40 different inversions have been recorded, compared to 34 in *D. paulistorum,* and 4 each in *D. tropicalis* and *D. equinoxialis.* In *D. willistoni,* the mean numbers of heterozygous inversions per individual vary in different populations from 0.8 to between 9 and 10 (in females); in *D. paulistorum* from 0.6 to 1.8; in *D. tropiculis* 0.14 and in *D. equinoxialis* 0.11. These facts confirm the hypothesis, advanced on the basis of comparative studies of different populations

of *D. willistoni,* that the chromosomal polymorphism present in a species or a population is positively correlated with the diversity of habitats occupied or exploited.

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FURTHER STUDIES ON *RANA PIPIENS* RACIAL HYBRIDS[1]

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PREVIOUS work on a common and widely distributed North American frog, *Rana pipiens* Schreber, has shown this species to be valuable for evolutionary studies (Moore, 1946, 1947, 1949a, 1949b). The geographic range of *pipiens* includes most of North America from northern Canada to Panama. With respect to a number of physiological characters, individuals from Florida, Louisiana, Texas and Mexico are better adapted to high temperatures than are those from Quebec, Vermont, Wisconsin and New Jersey. The adaptations so far investigated include embryonic temperature tolerance, rate of development, temperature coefficient for development, egg size, and type of jelly mass. It has been shown that northern and southern frog *species* differ from one another in most of these features, and in general, northern populations of *pipiens* differ from southern *pipiens* in the same way that northern species differ from southern species.

Data have also been collected on the " hybrids " resulting from crosses between individuals from different localities. The results are correlated with the temperature characteristics of the populations. If the cross is between an individual of a cold-adapted population (such as Vermont or Wisconsin) and a heat-adapted population (such as Florida or Texas), the zygote shows specific types of defects. The degree of defect is proportional to the latitudinal difference separating the parent populations. The most extreme abnormalities occur when the cross is between individuals from Vermont (or Wisconsin) and Florida (or Texas or Mexico). In these cases most or all of the embryos are so

[1] Aided by a grant from the Office of Naval Research.

abnormal that they die. The most extreme defects were observed in the cross Vermont ♀ × Mexico ♂. In this case cytolysis begins in the neurula stage.

These data were interpreted along lines suggested by Huxley (1939) and Muller (1942). It was thought that natural selection had led to the establishment of a gene complex providing for low temperature adaptation in northern populations and high temperature adaptation in southern populations. It was suggested that the developmental abnormalities in the hybrids are due to the inharmonious association of these two differently acting genetic systems. If this is a true explanation, the genetic differences that are the basis of adaptation are likewise the basis of an isolating mechanism, namely, hybrid inviability. Two sets of data indirectly support this possibility:

1. The magnitude of the difference in embryonic temperature adaptations between any two populations parallels the degree of abnormalities shown by their hybrids. This suggests that they may have a common basis.

2. The factors, presumably genes, that are responsible for the adaptations (such as temperature tolerance and rate of development) and the factors or genes that lead to hybrid malformation produce their effects at the same time. This also suggests that the genetic basis for adaptations and hybrid inviability is the same.

Recently I have secured material from Costa Rica and Colorado that allow a considerable extension of this work. I will not be able to report fully on embryonic adaptations in material from these localities since males alone were collected. The data on hybridization of these males with Vermont females are of such interest, and the possibility of getting additional material so remote, that the results already obtained will be given.

The interest of the Colorado material lies in the magnitude of the geographical distance separating that locality and Vermont. In the previously reported results on hybridization the developmental defects increased parallel with the latitudinal differences between parent populations. In interpreting the results emphasis was placed on environmental temperature in the northern and southern localities

as the selective force leading to divergence. It was not possible to rule out distance as an important factor although some data made this improbable. Material from Colorado, 1,700 miles west of the Vermont locality, would help to settle the point.

The situation with respect to the Costa Rica material is even more interesting. As previously mentioned, the most extreme hybrid defects observed have been in the Vermont × Mexico cross. If the observed trend continued, Vermont × Costa Rica should surpass all previous crosses in the degree of abnormality. There is, however, one interesting complication. The locality in Costa Rica where the *pipiens* were collected was not the hottest locality of those studied. The altitude was high and the year-round temperature is similar to that in Vermont during the summer. With this material it is possible to see whether the results are correlated with latitude or with environmental temperature. In previous experiments it was not possible to make this distinction.

Material

The Colorado males were collected August 18, 1949, at Musky Lake near Science Lodge, Boulder, Colorado, at an altitude of 8,300 feet. I am indebted to Professor K. W. Cooper for these *pipiens*.

The Costa Rica males were collected at Hacienda Moravia (9° 51′ N.; 83° 24′ W.) in Limon Province. The altitude is 3,700 feet. They were caught by K. W. Cooper and me on July 3 and 6, 1949. The climate is temperate and is probably similar to or slightly cooler than San José (altitude 3,700). The U.S.D.A. volume, "Climate and Man," gives 69.1° F. as the July mean at San José with the highest temperature ever recorded as 94°. The same source gives a July mean of 69.5° and highest temperature as 100° at Burlington, Vermont. It seems reasonable to conclude that environmental temperatures at Moravia are never higher than the summer temperatures at Burlington. The

air temperature at Moravia was 69.8° F. on July 6 at 2:00 P.M.

Moravia, Costa Rica, is further south than any other locality I know for *pipiens* either from published records or museum specimens.

Egg Size and Type of Jelly Mass in Costa Rica *pipiens*

On July 3 approximately 30 egg masses of *pipiens* were observed in a pond at Moravia. These were of the globular submerged type characteristic of New England populations. Northern and southern species of frogs frequently differ in the type of egg mass. The former have submerged globular masses and the latter surface films. It was clear from these observations at Moravia that, at least in this locality, southern *pipiens* do not differ from northern *pipiens* in the type of jelly mass. It may well be that this character does not show geographic variation (but see Moore, 1949b).

With respect to egg size, populations of *pipiens* from the southeastern United States resemble southern species in having small eggs. Northern populations of *pipiens* and northern species of frogs have large eggs. Eggs from Mexico, however, are of large size.

A few embryos were taken from eight clumps of gastrulae found at Moravia, preserved in 4 per cent. formaldehyde, and subsequently measured. The mean diameters for the eight groups varied from 1.92 to 2.11 mm. The mean of the sample means was 2.03 ± 0.05 mm., indicating an egg of large size. In comparing this value with data from other localities (Moore, 1949b) it must be remembered that these measurements were made on preserved early gastrulae and not on living uncleaved eggs, but I doubt if this makes a significant difference. Living gastrulae are slightly larger than uncleaved eggs, but since formaldehyde causes minor shrinkage the two probably cancel out. The Costa Rica *pipiens* in having large eggs resemble populations from Mexico, Wisconsin, New Jersey, Vermont and Quebec, and differ from those in Texas, Louisiana and Florida.

Hybrids

The male *pipiens* from Costa Rica and Colorado were brought to New York for study. They were kept at 16° C. and fed until used. Vermont females were the source of eggs. The methods of experimentation were the same as previously used (Moore, 1946).

Five crosses were made using Vermont eggs and both Costa Rica and Colorado sperm. Eggs of each female were divided into three groups. One group was fertilized by sperm of a Vermont male, the second by sperm of a Colorado male, and the third by sperm from a Costa Rica male. All groups were kept in a forced draft constant temperature incubator set for 19.6° C.

The development of Vermont ♀ × Colorado ♂ embryos did not differ from that of Vermont ♀ × Vermont ♂ embryos. So far as could be told this is a perfectly harmonious combination.

The development of Vermont ♀ × Costa Rica ♂ embryos affords a distinct surprise if one has anticipated a continuation of the trend shown by other population crosses. This combination, of a northern female and extreme southern male, resulted in nearly normal offspring. The five crosses gave essentially the same result, so they will be considered together. The Vermont ♀ × Costa Rica ♂ and Vermont ♀ × Vermont ♂ were identical in cleavage and blastula stages. The first difference was noted during gastrulation. The yolk plug of the Vermont ♀ × Costa Rica ♂ embryos was larger than in the controls. The Vermont ♀ × Costa Rica ♂ embryos first showed retardation in rate of development at the end of gastrulation, or during the neural plate stage. The anterior part of the neural plate of the Vermont ♀ × Costa Rica ♂ embryos was wider than in the controls. This was reflected in later development by the slightly enlarged anterior end of the Vermont ♀ × Costa Rica ♂ embryos. A slight shortening of the body was noticed. After hatching, the two groups were nearly identical in stage of development. This indicates that any difference in rate of devel-

opment was too slight to be detected. In one experiment gill circulation seemed somewhat abnormal, and in another a few of the Vermont ♀ × Costa Rica ♂ embryos died as neurulae. For the most part perfectly healthy tadpoles were formed.

The point of exceptional interest is the slight abnormalities observed in the Vermont ♀ × Costa Rica ♂ embryos. When a Vermont ♀ × Texas ♂ or Vermont ♀ × Mexico ♂ cross is made, the embryos are so defective that most or all die shortly after hatching. Nothing like this is observed in the Vermont ♀ × Costa Rica ♂ hybrids. Instead, they resemble more the hybrids from regions closer together, such as New Jersey and Louisiana.

Discussion

The data on Colorado and Costa Rica *pipiens* can readily be explained by the hypothesis of the interrelation between embryonic adaptations and hybrid inviability. When crosses are made between individuals from markedly different temperature regions, the hybrids are defective, and the degree of defect correlates, at least in a rough way, with the magnitude of the temperature difference between the regions. In both Colorado and Costa Rica it is probable that *pipiens* is not subjected to temperatures higher than those encountered in the northeastern United States. In Costa Rica they are never subjected to temperatures as low as those encountered in the northeastern states. It might be surmised that embryonic adaptations, such as temperature tolerance and rate of development, are identical in Vermont and Colorado and nearly the same in Vermont and Costa Rica. This must, of course, remain an open question until the actual measurements are made.

The normalcy of the Vermont ♀ × Colorado ♂ embryos shows that wide geographical separation is not necessarily associated with racial incompatibility. The distance between these two localities is 1,700 miles. On the other hand, Vermont and Florida crosses give very abnormal hybrids, yet the difference between these localities is only 1,400 miles.

The results with Costa Rica *pipiens* add some support to the hypothesis that hybrid inviability in population crosses is associated with temperature adaptation. The Vermont ♀ × Costa Rica ♂ cross is uniting individuals from localities 3,500 miles apart (shortest land distance). The Costa Rica locality is much farther from Vermont than is the Florida (1,400 miles), Texas (1,800 miles) or Mexico (2,200 miles) localities previously studied. The Florida, Texas and Mexico environments are warmer than Vermont, and their *pipiens* populations differ in embryonic adaptations. The Costa Rica locality is similar to Vermont with respect to mid-summer mean and maximum temperatures. It differs considerably in lacking seasonal temperature changes, since the January and July means are nearly the same.

The morphological defects resulting from the Vermont ♀ × Costa Rica ♂ cross are very slight. This result might be explained along the following lines. In Costa Rica natural selection is not promoting the establishment of genotypes of the high temperature sort found in Florida, Texas and Mexico. Instead, both Costa Rica and Vermont populations have similar genotypes with respect to embryonic temperature adaptations. Their union in a hybrid does not result in gross abnormalities.

It is interesting to speculate on the possible origin of the Costa Rica population. These highland forms may have differentiated from a Central American lowland type of the kind previously described for northeastern Mexico. If this is the case, the development in Costa Rica of a type similar to that found in northeastern United States is an example of parallelism. A situation of this sort calls to mind the independent development of alpine ecotypes in different mountain ranges (Turesson, 1925, 1931) and the phenomenon of bipolarity shown by many marine organisms. As another possibility, a cold-adapted type could have migrated south from the Rocky Mountain region and populated those areas where it was best suited to survive. If this is so, the highland populations of *pipiens* in Mexico and Central America never were of the warm-adapted type.

This second possibility could be tested by comparing lowland and highland forms, preferably in adjacent regions. In eastern Mexico this could be done readily since *pipiens* occurs in the tropical lowlands at the base of the Sierra Madre Oriental and at high elevations in the mountains themselves.

Summary

The data presented in this paper are a continuation of a study of geographic variation of adaptations in *Rana pipiens* Schreber and of hybrids arising from interpopulation crosses.

The eggs and jelly mass of Costa Rica *pipiens* are described. They are of the northern, cold-adapted type.

Hybrids resulting from the cross Vermont ♀ × Colorado ♂ are normal.

Hybrids resulting from the cross Vermont ♀ × Costa Rica ♂ have minor defects. This result is discussed in light of the previous cases of defective hybrids arising from crosses of northern and southern *pipiens*.

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AN ESTIMATE OF THE NUMBER OF LOCI IN THE X CHROMOSOME OF *D. MELANOGASTER*[1]

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THE number of loci in the X chromosome of *Drosophila melanogaster* has been estimated by various means. The number of loci is deduced to be 660 if, following Muller's (1926) reasoning, the minimum crossover distance measured were 0.1 units and the length of the crossover map taken as 66.0. Another estimate may be made using the number of separate bands in the salivary gland X chromosome. The number of bands, about 1024 according to Bridges (1935, 1938), suggests a lower limit for the number of loci. Catcheside and Lea calculated the number of loci capable of showing a recessive lethal mutation to be 261 or 838 if the locus diameter is 6 or 4 mμ, respectively, on the assumption that the probability of a lethal mutation occurring when an ionization is produced in the locus is unity (see Lea, 1945). Demerec (1934) arrived at 500 as the number of loci in the X chromosome by dividing the frequency of X-ray induced mutational changes in all loci by the average frequency of change per single locus. Gowen (in the "Discussion" following Demerec, 1934) estimated the number of X chromosome loci to be 1200 from his experiments. From these studies it seems likely that the number of X chromosome loci lies between 500 and 1200. Using a method quite different from those already mentioned, Wright arrived at 289 as a minimum estimate of the number of loci on the third chromosome of *D. pseudoobscura* capable of mutation to recessive lethals, on the assumptions that all the loci producing lethals have the

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same mutation rates and that recessive lethals on this chromosome have no deleterious effects in heterozygotes (Dobzhansky and Wright, 1941). Consideration of the number of bands in this chromosome as seen in salivary gland cells suggested 1000 loci as a rough maximum estimate (Dobzhansky and Wright, 1941).

This paper is an attempt to arrive at an estimate of the number of X chromosome loci in *D. melanogaster* by another method. This consists of dividing the total number of breaks produced in the X chromosome of viable sperm by the number of mutations produced after breakage at a single locus. The assumptions involved in this calculation will be presented together with the evidence affecting their validity.

According to Demerec (1934), 69 of 82 (84 per cent.) tested mutants occurring at 22 loci scattered throughout the length of the X chromosome were lethals or deficiencies. Gowen (in the "Discussion" following Demerec, 1934) calculated that 75 to 80 per cent. of the mutations produced in his experiments were lethals. Had all the detected mutants been tested, and these values remained unchanged, the chance that an observed mutation would be due to a lethal or a deficiency is about 75 to 84 per cent. It is commonly agreed that the majority of X-ray-induced recessive lethals are deficiencies (Demerec, 1937). Since the great majority of recessive lethals are produced at points where the chromosome has been broken (see Lea, 1945; Herskowitz, 1946; Fano, 1947) and because the production of a deficiency necessitates chromosomal breakage, it would seem that roughly 75 to 84 per cent. of all detected mutations involve chromosomal breakage. This would indicate that if the total number of mutants at a typical locus were divided into the total number of viable breaks produced, the number of loci would be underestimated by 16 to 25 per cent. After correcting for the underestimate a fairly good estimate of locus number might be obtained.

It would seem that the best estimate of number of loci would come from the study of a locus having the following

properties: that it breaks and mutates with average frequency; that the proportion of mutations connected with breakage to other types of mutation is typical; that mutations connected with breakage are characterized by unique phenotypes; and that all the mutations connected with breakage are detected. This would permit calculation of number of loci directly. The white locus seems to possess these qualities. The X chromosome probably breaks at the white locus with the same frequency that it breaks at other loci since the chance of X-ray breakage is the same for equivalent lengths of each chromosome (see Catcheside, 1948, for discussion). That a break results in mutation just as frequently at the white locus as at other loci is consistent with the available evidence that X-ray induced lethals are distributed along the chromosome at random, and that, in so far as the genes studied are representative, the rate of visible mutations induced at different loci is sensibly identical (see Catcheside, 1948). A great proportion of the mutations to white would seem to involve a deficiency since back mutation is rare or absent (Timoféeff-Ressovsky, 1933; Lefevre, 1949). On the other hand, since there is no detectable difference in the mutation rates of wild-type and seven other alleles, not including white, to white or to other alleles (Fryer and Gowen, 1942), it is reasonable to believe that these alleles are not deficiencies in the usual sense. If white is the only allele that is considered a deficiency, then according to the calculations presented above about 75 to 84 per cent. of the mutations at this locus should result in the white phenotype. Timoféeff-Ressovsky (1933) obtained an experimental value of 71 per cent. (47/66). It seems reasonable, therefore, that the proportion of deficiencies produced at the white locus is approximately the same as at other loci and that most of the mutations to white are produced at points of breakage. In addition to being unique, the white eye phenotype will seldom be missed in mutation studies.

If only one half the breaks at the white locus bear mutations our calculations would overestimate the number of

loci by 100 per cent. However, according to Lea (1945), 15 to 20 ionizations are required to produce a break in the chromosome and the probability is greater than 0.3 that a recessive lethal mutation will occur when a single ionization is produced in a gene. It would seem, therefore, that the probability for a break involving a locus to bear a lethal-deficiency mutation is very close to unity so that the number of loci overestimated is negligible. If the probability is close to unity that a lethal or a deficiency mutation is produced when breakage involves a gene locus then almost all breaks at the white locus should produce the white phenotype leaving most other mutations produced at this locus apparently unassociated with breakage. Accordingly, the great majority of mutations to alleles other than white should represent gene mutations (Stadler, 1932).

Since we have seen that the mutability at the white locus is not detectably different either quantitatively or qualitatively from other loci studied, it is reasonable to assume that this locus is suitable to represent, in size, one locus in the chromosome.

Accepting the above interpretations as being essentially correct, one may obtain an estimate of the number of loci in the X chromosome by dividing the total number of breaks produced in viable sperm by the number of observed mutations to white. Estimates made in this manner are presented in Table 1. The number of chromosome breaks produced in viable sperm for different doses of X-rays determined by three different methods by Catcheside and Lea and by Herskowitz (see Catcheside, 1948, for discussion) are in substantial agreement. Accordingly, the number of X chromosome breaks per viable sperm for any given X-ray dose was calculated employing the procedure and Table 42 (p. 169) given in Lea (1945). The data for the X-ray induced mutation rate to white were obtained from Timoféeff-Ressovsky (1933) and Bonnier and Lüning (1949). The number of loci calculated using data from treatments with different doses of X-rays varies from 597–890. When each

of these values are weighted by the number of X chromosomes tested the weighted mean is 726 loci.

If white is more mutable than the average locus or if not all mutations to the white phenotype are recessive lethals or deficiencies, the number of loci is underestimated. On the other hand, if some mutations to intermediate alleles are lethal or deficient the locus number is an overestimate. Undoubtedly, this estimate will need revision when further

TABLE I

Summary of Data Used to Calculate the Number of Loci on the X Chromosome

Dose r	Number mutations to *w*	Number X chromosomes tested	Author	Number X chromosome breaks per 10^4 viable sperm (Lea, 1945)	Number loci estimated
727	40	402,005	Bonnier and Lüning	594	597
1,454	36	244,542	Bonnier and Lüning	1,092	744
2,181	28	162,064	Bonnier and Lüning	1,540	890
2,908	22	92,409	Bonnier and Lüning	1,950	820
4,800	47	134,000	Timoféeff-Ressovsky	2,920	832
Weighted mean					726

studies are made of mutation at the white and other loci and of the number of breaks in viable sperm.

Summary

By dividing the total number of viable breaks by the number of mutations occurring at breakage points at a single typical locus, one can obtain an estimate of the number of loci present. Assuming the white locus of *D. melanogaster* to be a typical one and that all viable breaks involving this locus result in the white phenotype, the number of loci on the X chromosome is calculated to be approximately 726.

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MUTATIONS AND MUTAGENIC AGENTS IN BACTERIA

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As previously reported, mutation to penicillin or streptomycin resistance can be induced in *Micrococcus aureus* by growing the organism in nutrient broth and in certain synthetic media which have been irradiated with ultra-violet light (Stone, Wyss, Haas, 1947) or treated with hydrogen peroxide (Wyss, Stone, Clark, 1947; Wyss, Clark, Haas, and Stone, 1948).

Stone, Haas, Clark, and Wyss (1948) showed that these mutations result from an increase in mutation rate and are not due to selective action in favor of pre-existing mutants in the culture. The penicillin and streptomycin resistant mutants grow no faster than the original strains in either irradiated or unirradiated media. Mixtures of normal and mutant *Micrococcus aureus* exhibited the same ratio after growth in irradiated and unirradiated media that they had before growth. Penicillin resistant mutants had no more resistance to streptomycin than did their original parent strain. When these penicillin resistant mutants were grown in irradiated broth or hydrogen-peroxide-treated broth the treatment induced mutation to streptomycin resistance in these at the same rate as that induced in the normal strain. The same characteristics hold for the streptomycin resistant mutants.

In attempts to characterize the mutating principle, and to determine the method of its production by ultra-violet irradiation or hydrogen peroxide treatment, we have investigated some of the physical properties of treatment of the substrate, and of the treated substrate itself.

It was found that the mutating principle could not be removed from irradiated media by charcoal, but successive treatment with bacteria would remove it completely as shown in Table I. In these experiments a sample of irradiated broth was inoculated with bacteria; the bacteria were filtered out, and the filtrate reinoculated with fresh bacteria. This procedure was repeated several times for the same sample of treated broth. The number of antibiotic resistant mutants was determined for each inoculation by

TABLE I

Removal of Mutating Principle from Irradiated Broth by Repeated Inoculations and Filtration of Micrococcus Aureus

Filtration Treatment	Irradiation Treatment	Penicillin Resist. Mutants	Streptomycin Resist. Mutants
Before filtering	Unirradiated	32.5	101
	Irradiated	65.3	210
After filtering once and re-inoculating	Unirradiated	31.2	86.8
	Irradiated	54.0	130
After filtering and re-inoculating twice	Unirradiated	34.9	72.5
	Irradiated	8.5	69
Inoculated after filtering twice [1]	Unirradiated	31.8	74.6
	Irradiated	52.4	190
Irradiated broth, not filtered. Two hours between radiation and inoculations. [2]		56.0	182

[1]Filter control shows how much mutating principle filtering removes.
[2]Age control shows how much mutating principle is removed during period between irradiating and final inoculation due to decay of the principle.

testing a sample just before each filtration. It should be noted that the incidence of mutation was returned to near normal after three or four of these inoculations. The same effect is shown by using extremely large inocula (several million organisms per ml.) in irradiated broth. In general, the larger the inoculum the smaller the relative increase in mutant frequency due to induced mutation. The mutagenic qualities disappear after four days standing in the dark in tightly closed containers.

It has been well established that in direct irradiation the most effective wave-lengths for producing mutations are in the 2537–2650 Å region. However, in irradiation of the substrate, the most effective wave-lengths are in the range of 2000 Å or below. This test was first attempted using a quartz monochrometer, but the intensity of the short wave-lengths emerging from the quartz system was too low to be effective. As substitute a series of ultra-violet transmitting filters was used. A filter which would not transmit below 2170 Å caused only a very small increase in mutation rate. As a result of the dependence on low wave-lengths, only a quartz ultra-violet lamp is effective in substrate irradiation. The operating temperature of the lamp will affect the results; the lower the operating temperature of the lamp the greater the output of the very short wave-lengths, and thus the higher the mutation rate.

The temperature of the substrate during irradiation will also have an effect on its subsequent mutagenicity. This effect is shown in Fig. 1 where incidence of mutation is plotted against temperature. As the temperature of the substrate during irradiation is increased, more peroxide is found and the incidence of mutants in bacterial cultures grown therein increases. At a temperature between 60° C. and 70° C. the water vapor above the substrate is increased to such an extent that it absorbs an appreciable amount of the radiation, and the mutagenicity and peroxide formation decrease. It is probable that the mutagenic principle is destroyed rapidly at this temperature since heat has been shown to destroy it.

The induction of mutation in various other organisms by means of treated substrates shows that it is a general phenomenon and not peculiar to a few forms such as *Micrococcus aureus*. Wagner, Haddox, Feurst, and Stone (1950) induced biochemical mutations in *Neurospora crassa* by this method. We have treated various strains of *Escherichia coli*, an organism differing from *Micrococcus aureus* in many respects, and with a mutation system of which much is known (that of resistance to bacteriophage). It has the additional advantage of growing in a simple synthetic

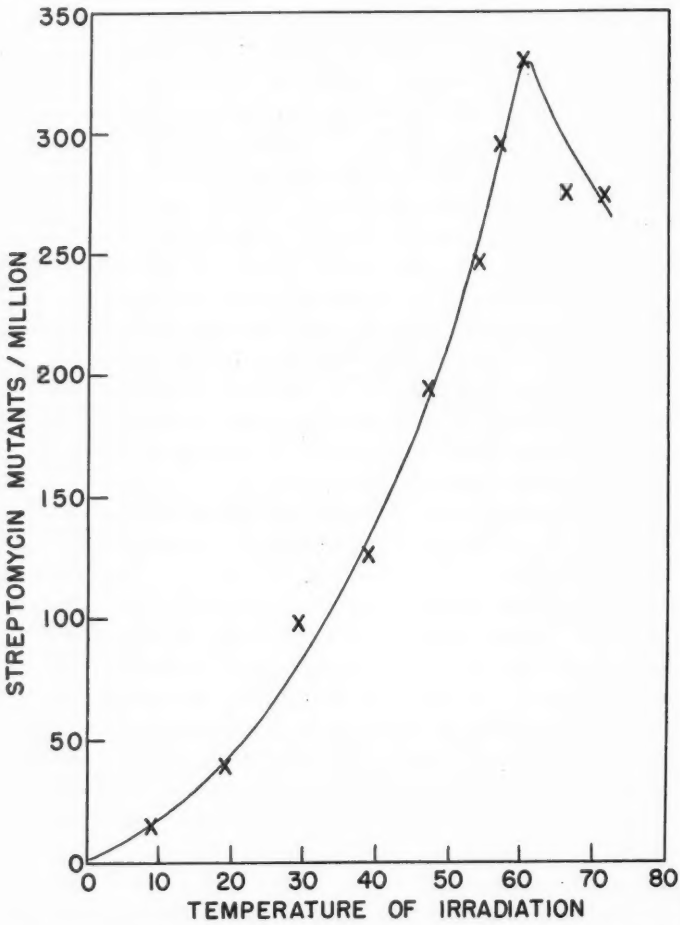

FIG. 1. Effect of temperature of substrate during radiation on the production of the mutating principle.

medium, thus permitting the ready detection of biochemical mutants.

In these tests two strains of *E. coli* were grown either in treated or untreated substrates, then tested by the soft agar layer technique for the number of phage-resistant colonies developing in the presence of bacteriophage T–1. The results obtained confirm our earlier findings of drug-resistance

in *Micrococcus aureus* in that the number of phage resistant *E. coli* is much greater among those grown in irradiated or hydrogen-peroxide-treated samples than in the untreated controls (Table II). The treatment increases the incidence of mutation to phage T–1 resistance above the spontaneous rate in both of the strains used here. Strain 58–161 is a mutant of *E. coli* K_{12} and is one of the organisms used by Lederberg and Tatum (1946), Lederberg (1947), and Tatum and Lederberg (1947) to demonstrate genetic recombination in bacteria. Strain B is a strain of *E. coli*

TABLE II

Induced Mutation to Bacteriophage T–1 Resistance by Treatment of the Substrate

(Entries are in resistant mutants per million)

Substrate treated by irradiating with ultra-violet light

	Broth irradiation time in minutes			
E. coli strain	0	20	40	60
Strain B	2.4	2.9	90	31
Strain 58–161	1.8	...	35	28

Substrate treated with hydrogen peroxide for 60 minutes

	PPM H_2O_2 used in treatment		
E. coli strain	0	5	10
Strain B	1.3	2.9	6.9
Strain 58–161	1.8	16	46

which has not shown genetic recombination. It is important to note here that, although the mutation rates are different for the two strains, an increase in the mutation rate is effected by treated substrate in both the non-recombining and recombining strains. This indicates that the genetic constitution is similar in both types of strains as far as can be judged by this similar increase in mutation.

The same tests for differential selection between normal and mutant strains have been used with these mutants that were performed earlier with *Micrococcus aureus* (Stone, Haas, Clark, and Wyss, 1948). It was found that in all

cases any possible selection would have been against the mutant rather than for it. Some of the resistant mutants tested grew more slowly in both irradiated and unirradiated broth than did the parent strain, but none grew faster.

A number of experiments demonstrate that the mutagenic substances produced in the medium by ultra-violet irradiation have a maximum point beyond which further irradiation will produce no more mutagens and, in fact, seems to destroy those already produced. This effect is apparent in Table II in the broth irradiated for 60 minutes for both *E. coli B* and strain 58–161, if one follows through the table from 0 to 60 minutes irradiation time. The effect is not present in substrates treated with usable concentrations of hydrogen peroxide. It was found that while the mutagenic power of broth increased with the time of irradiation up to 40 minutes ultra-violet irradiation, the mutagenic activity decreased slowly. The same effect is found when growing *Micrococcus aureus* in broth irradiated for different periods of time. The effect could be accounted for by assuming either that there is a maximum amount of substance in the medium which is acted upon by the irradiation or by products of the irradiation such as hydrogen peroxide, or that there is a maximum rate or concentration of products formed in the medium by our source of ultra-violet. In either case prolonged irradiation destroys the mutagens.

Both phage resistant and non-resistant colonies of *E. coli* 58–161 were tested for biochemical mutations. Out of 432 phage resistant colonies tested 61 were found to have biochemical deficiencies, while out of 100 colonies of non-resistant strain, 58–161, no biochemical mutant was found. This indicates that certain metabolic changes are necessary for, or at least often accompany, some types of bacteriophage resistance. The deficient phage resistant mutants were tested for amino acid, vitamin and purine and pyrimidine requirements. Two were found to have a requirement for proline, and the remainder were found to require either a mixture of certain amino acids or some unknown factor

present in yeast extract. We find that the use of bacteriophage in this manner is an excellent method for the selection of biochemical mutants, for it is both rapid and simple. As all the biochemical mutants isolated must be virus resistant, the method limits the types of mutants obtainable and for this reason many desirable ones would undoubtedly be missed.

The incidence of mutants in *E. coli*, strain 58–161, due to spontaneous mutation to phage resistance was determined on media of different complexity (Table III). It was found

TABLE III

Incidence of Mutation Due to Spontaneous Mutation to Bacteriophage T–1 Resistance in Strain 58–161 Cultured in Various Media

(Entries are T–1 resistant mutants per million cells)

History of Culture	Media in which analyzed cultures were grown		
	Hershey broth	McLeod's + asparagine	Ammonium lactate broth
From nutrient agar slant to McLeod's broth	68	333	404
From nutrient agar slant to NH_4 lactate broth	215	448	1,384
From McLeod's agar slant to McLeod's broth	80	85	200
From McLeod's agar slant to NH_4 lactate broth	139	318	414
From nutrient agar slant to Hershey broth (control)	74	...	...

that the more complete the medium, the lower the incidence of mutation. In the tests the incidence of mutation to bacteriophage resistance is lowest when the organism is grown in complete medium (Hershey and Rotman, 1949), and greatest when the organism is grown on ammonium lactate medium. It is intermediate when grown on McLeod's (1940) synthetic medium, which is intermediate in complexity. These facts suggest that mutations are more prevalent when there are more steps in the biochemical syntheses; there is more chance for the organism to make an error in gene reduplication.

Whitaker (1942) showed that visible light would reverse the lethal effects of ultra-violet irradiation of Fucus eggs. Recently this work has been confirmed and extended to microorganisms by Kelner (1949a, 1949b) and by Novick and Szilard (1949). Kelner finds that visible light of wave-lengths under 5100 Å will cause some recovery of *Streptomyces griseus, E. coli, Penicillium notatum,* and *Saccharomyces cerevisiae* from the lethal effect of ultra-violet irradiation. Novick and Szilard (1949) found that visible light also reduces the number of mutants induced by the ultra-violet irradiation. Tests of our material show that visible light reverses the lethal effect of ultra-violet irradiation on *Micrococcus aureus,* though not as great an

TABLE IV

Direct Effects of Various Amounts of Ultraviolet and Visible Light on Micrococcus Aureus

Secs. of uv radiation	Cells surviving treatment by U.V. and visible light		
	No visible light	20 min. visible light	120 min. visible light
0 Sec.	229 million	249 million	16.8 million
30 Sec.	240,000	31.9 million	10 million
60 Sec.	250	290	560
90 Sec.	520	400	160

effect was noticed here as in Kelner's work. It will be observed from Table IV that the visible irradiation itself has a strong lethal action when used in large amounts. Also when a certain amount of ultra-violet radiation is used (above 30 seconds in our experiments) there is little if any reversal of the lethal effects by white light. In other experiments, *Micrococcus aureus* was inoculated into nutrient broth which had been irradiated with ultra-violet for a three-hour period. Broth irradiated for this length of time will usually reduce the number of bacteria inoculated into the culture to about 20 to 30 per cent. of its original value. The culture was then split into two portions and one part was stored in the dark while the other was irradiated with visible light for various lengths of time. This treatment

with visible light reduced the lethal effects of the nutrient broth which had been irradiated with ultra-violet. The effect is somewhat smaller than is the case with direct ultra-violet irradiation, but it is still apparent.

In both of these experiments visible light radiation controls were run. An unirradiated broth sample was inoculated with bacteria and then irradiated with visible light. These controls showed that visible light produces a lethal effect of its own as well as a reversal of ultra-violet injury. This lethal effect becomes quite large with excessive exposure to visible light and undoubtedly accounts for the fact that very little lethal reversal is seen in ultra-violet treated samples which have received long exposures to visible radiations. In experiments of this type it was necessary to determine the optimal amount of visible light to be used in the experiments. For example, 20 minutes' exposure to 14,000 foot candles was the maximum amount of visible light that could be used without producing a marked lethal effect on *Micrococcus aureus;* at 2000 foot candles there was very little killing with 60 minutes' irradiation. Organisms removed from the irradiated broth and suspended in buffer prior to irradiation with white light gave results similar to those exposed to the light while suspended in the broth. Yet other experiments showed that visible radiation had some effect on the mutagenic action of a medium which had been irradiated with ultra-violet light. In these experiments numerous replicate tubes of both nutrient broth and Gladstone's synthetic media (Gladstone, 1937) were irradiated with various combinations of ultra-violet, visible light, and infra-red (Table V). All tubes were inoculated, after irradiation, with the same amount of a log phase culture of *Micrococcus aureus* and after incubation for three hours the lethal effect and the effect on the incidence of mutation to streptomycin resistance were measured by the usual procedures. Results showed that irradiation of the nutrient substrate with ultra-violet followed by visible light reduced both the lethal effect and the mutation rate to streptomycin resistance from that shown in the sample irradiated with

ultra-violet alone. In nutrient broth these reversal effects were small but always consistent. The killing reversal was not as consistent in Gladstone's synthetic medium; however, the induced mutation to streptomycin resistance was always decreased. Irradiation of the media with visible light alone always produced a slight rise in the incidence of mutations, even though it reduced the number of mutations induced by ultra-violet irradiated broth.

TABLE V

Effect of Media Irradiated with Various Combinations of Ultra-violet, Visible, and Infra-red on the Growth and Incidence of Mutation Rate to Streptomycin Resistance in Micrococcus Aureus

(Mutant entries are mutants per million)

Treatment of Media	Nutrient broth		Gladstone's broth	
	Growth	Streptomycin mutants	Growth	Streptomycin mutants
Unirradiated control ...	118 M[1]	720	98 M	5.7
Irradiated with visible light	131 M.	1,500	13 M	45.4
Irradiated with ultra-violet	59 M	8,100	83 M	180
Irradiated with ultra-violet followed by visible.....	71 M	7,700	16 M	103
Irradiated with ultra-violet followed by infra-red	50 M	350	9 M	67
Irradiated with infra-red	14 M	112	38 M	25
Irradiated with ultra-violet and heated at 70°C for one hour ...	122 M	3,500	105 M	46.5

[1] M = million.

Irradiation of medium with infra-red alone reduced the incidence of spontaneous mutations and also caused severe killing of organisms subsequently inoculated therein. Irradiation with ultra-violet followed by infra-red caused severe killing and a decrease in number of mutants even below the normal frequency. Not all the effect resulted from heat since the samples never reached a temperature higher than 50° C. during infra-red irradiation. When an

ultra-violet irradiated sample was heated at 70° C. for one hour it still gave a mutation rate considerably higher than the spontaneous rate, although much lower than the ultra-violet irradiated sample.

Part of the effect of visible light in reducing the lethal and mutagenic effect of both the direct and indirect action of ultra-violet light may lie in the destruction of hydrogen peroxide, unstable organic peroxides, and other types of unstable molecules. Luria has called to our attention the fact that the effect of hydrogen peroxide can not account for the reactivation of phage which occurs only in a suitable host. But the evidence indicates that the phenomenon of phage reactivation is due to some form of recombination rather than mutation. In a number of tests, the breakdown of peroxides by visible light has been followed by chemical and physical means. The destruction of other possible lethals and mutagenic agents has not been demonstrated except by the survival and mutation test. Hydrogen peroxide acting directly on *Micrococcus aureus* cells in the absence of an organic substrate does not increase the mutation rate measurably, although it does increase that of *Neurospora,* according to Dickey, Cleland, and Lotz (1949) and Wagner, Haddox, Fuerst, and Stone (1950). Most of the mutagenic activity of organic substrate treated with hydrogen peroxide is destroyed by the addition of catalase (Wyss, Clark, Haas, and Stone, 1948). Presumably organic peroxides or unstable radicals are in equilibrium with hydrogen peroxide or are otherwise destroyed by catalase in this case as well as in irradiated broth. Dickey, Cleland, and Lotz (1949) showed that certain very reactive organic peroxides are mutagens, and suggested that this activity is due to the formation of free radicals by these substances. They did not study the production of organic peroxides or other products formed by irradiation.

Lea (1947) reviewed the evidence that short-wave irradiation of water produced free radicals and that organic materials are decomposed or, if sufficiently concentrated, may act as protective agents for other substances against

the free radicals. Lea, in his development of the biological effects of radiation, states:

> It is possible that some actions of radiation of biological significance are due to this separation of electrical charge, but in most cases it is more plausible to attribute it to chemical change resulting from the ionization. For, *when an atom is ionized the molecule of which it is a part almost certainly undergoes chemical changes* (italics in Lea).

He points out that a second effect of short-wave radiation, possessed also by ultra-violet, is *excitation*. This excitation is not much less effective than ionization in causing chemical change, although large organic molecules are more resistant to its effect.

In the same year Stone, Wyss, and Haas (1947) proved that ultra-violet light caused changes resulting from this excitation in the irradiated substrate, which later caused an increase in mutation rate in bacteria inoculated into this substrate. The work of Auerbach and Robson (1944; see review by Auerbach (1949) for full discussion and references) proved that mustard gas and some related chemicals can induce mutation. Stadler and Sprague (1936) proved that ultra-violet induced mutations in maize. The work of our group has shown that irradiation with ultra-violet can produce chemical mutagens even from simple amino acids. Therefore, some part of the effect of direct irradiation with ultra-violet probably is through such secondary mutagens.

For a long time after Muller (1927) demonstrated the effect of X-rays on genes and chromosomes, the effect was usually described through the target theory, which assumed that a direct hit and effect of the ionization on the chromosome was both necessary and sufficient to produce the genetic effects.

The chemical mutagens produced by ultra-violet have demonstrated that such a direct hit is not necessary for genetic effects. Bonnier and Lüning (1949) and Bonnier, Lüning and Perje (1949) have demonstrated that in addition to the direct effect, there is a slight indirect effect of X-rays on the mutation rate. Presumably this is related to the production of chemical mutagens by irradiation that

we have demonstrated more directly. Thoday and Reed (1947), Thoday (1949), Giles and Riley (1949), Riley and Giles (1950), Baker and Sgourakis (1950) and Faberge (1950) have demonstrated that oxygen is involved in the production of most genetic changes so that ionization is not sufficient to produce genetic changes under all conditions.

At present the relative importance of indirect genetic effects where the immediate radiation-induced change is outside the chromosome, and the relation of oxygen and hydrogen peroxide and free radicals to mutations and chromosome breakage are unsolved but developing problems.

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HYBRIDS OF PIGEON BY RING DOVE[1]

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INTRODUCTION

HYBRIDIZATION of domestic pigeons (*Columba livia*) with domestic ring doves (*Streptopelia risoria*) has been carried on at this laboratory for about 25 years. Numerous problems are presented by this cross, and with the passing years emphasis has shifted from one aspect to another. It is our purpose in this paper to bring the varied phases together as well as possible, and to gather all available key references pertaining to this cross.

The first concern was to obtain the hybrids. Natural mating is not very difficult to obtain after isolating male pigeons and female ring doves, though it is impossible to use some pigeons, especially if very pugnacious or large. On the other hand, one can seldom get natural mating of the small male ring dove with a female pigeon. The reason is not only the greater size of the pigeon; she appears to require a more boisterous suitor, as a rule. Size differences are probably a major obstacle to fertility, however, once matings are established. For example, eight matings of male pigeons by female doves produced 104 eggs, all infertile, while by contrast seven other matings of the same sort produced a total of 147 eggs, with 94 per cent. fertility. Twelve matings of male doves by female pigeons gave a

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total of 304 eggs, all infertile, while two other such matings produced 63 eggs with about 10 per cent. fertility. (Broken eggs, etc., are excluded from these calculations.) Pigeons of rather mild disposition and small size have been used preferentially. They were mongrels or of various pure breeds such as Tumblers and Tipplers and Owls. The resulting hybrids consequently have differed strikingly among themselves.

Painter and Cole (1943) have reviewed the problems of the hybrids' sex ratio. The cross of male pigeon by female dove has given less than 3 per cent. female hybrids at ages when gonads can be seen readily. These few female hybrids have been small, delicate, short-lived, and without sexual activity. By contrast, the reciprocal cross has readily produced viable female hybrids, and they have shown a little sexual behavior. In the former cross, a very high embryonic mortality is prominent. A large proportion of the dying embryos are at the early "blood ring" stage (Zeimet, 1931; Willoughby, 1934). Painter and Cole's studies of chromosome complexes of hybrids, including such dying embryos, indicate that the unusual sex ratio can be accounted for by differential mortality. Since these authors have reviewed most of the literature and discussed the theoretical problems we shall add only one comment at this point: some data presented by Riddle (1925) (*e.g.*, 11 consecutive eggs from one mating produced males) seem difficult to explain merely by the effect of differential mortality. However, as will be indicated in connection with sex-linked factors, there is no evidence in our data for sex reversal, the alternative preferred by Riddle.

Extensive effort was made to breed the hybrids, but few offspring were obtained from them. Again, these backcross offspring were repeatedly mated with doves in a fruitless attempt to continue the experiment. The results of these tests are reported here for the first time.

Phenotypic variation among the hybrids was noted by earlier investigators, and some of the early hybrids in this laboratory have been discussed from this viewpoint by Wentworth (1924). However, the bulk of the data will be

TABLE I

LIST OF MATINGS WHICH PRODUCED OFFSPRING WITH RECORD OF PLUMAGE

MATING	MALE	FEMALE	OFFSPRING a	OFFSPRING b
D4	1710H Tumbler mongrel	1808A blond ring dove		2
D5	1602H mongrel, "cream pied"	1561A " " "	2	
D29	1583M " dun	1807A " " "		1
D48	1863A Tumbler, yellow	D24A " " "		2
D69	1583M mongrel, dun	1416A white " "		2
D93	1710H Tumbler mongrel	D51C2 blond " "	1	
D107	2141A " Black Baldhead	D2G " " "	1	
D108	2140A " " "	D51D2 white " "	3	12
D110	1964C mongrel, ash	D2H blond " "	2	10
D118	1602H " , "cream pied"	D2K " " "	2	
D123	2139A Tumbler, Blue Baldhead	D26A " " "	1	
D128	1710H " mongrel	D99A white " "		1
D130	2141A " Black Baldhead	D73H blond " "		2
D147	2121A Tippler, grizzle	D2G2 " " "		2
D148	2022A " "	D59S " " "	5	14
D152	2081F mongrel, ash	D80M " " "	1	
D165	1710H Tumbler mongrel	D16A white " "		2
D183	2266.1 Brander, bronze	D115L blond " "	2	
D196	D130P hybrid, black	D144B " " "		1
D197	D130H " "	D70E " " "	1	
D199	2121A Tippler, grizzle	D80Q " " "		2
D210	1710H Tumbler mongrel	D155:2 white " "		1
D234	D110E3 hybrid, cream	D83A blond " "		2
D251	2218B mongrel, black	D150F " " "	1	
D262	D110R3 hybrid, blond	D121A " " "	1	1
D311	2414.2 Owl, ash	D212L " " "	1	7
D770	D204A blond ring dove	2530B Tippler, grizzle	2	
E21	2664J Archangel, bronze	D155:44 blond ring dove	2	8
E321	2596B mongrel, black silky	D155:369 " " "	2	2
E375	2634M2 " "	D155:423 " " "	2	
E387	E21Y hybrid, dun	D155:424 " " "		1
E437	2854.2 Chinese Owl, dun	D155:487 " " "	1	
*E455	D155.186 blond ring dove	2856E Tumbler, Blue Baldhead		4
E485	2855.1 Tumbler, Blue Baldhead	D155:505 blond ring dove		1
E496	2596B mongrel, black silky	D155:589 " " "	1	2
E507	2853.2 Turbit, red	D155:601 " " "		4
E511	2854.1 Chinese Owl, dun	D155:613 " " "		1
E522	2854.2 " " "	D155:582 " " "		1
E564	2854.2 " " "	E84.13 white " "	1	5
E635	E21F2 hybrid, dun	D155:676 blond " "		1
E738	E21Y " "	D155:634 " " "		1
E920	E21Z " "	D155:885 " " "		1
E992	2854.2 Chinese Owl, dun	D155:999 " " "		1
G235	E938S mongrel, almond	D155:949 " " "		1

The mating numbers in the left column are in serial order. Identifying numerals of males and females are made up of parents' mating number followed by a letter giving serial order of egg or by a decimal indicating purchase. (a): young which died in juvenile plumage; (b): matured.
* By artificial insemination.

presented and analyzed genetically here. Three principal aspects are involved: (1) the behavior of genes from the pigeon in the hybrid milieu; (2) the use of sex-linked genes to identify genetic sex of the hybrids; and (3) observations on segregation in the backcross.

A list of all the matings which produced offspring old enough for plumage description is given in Table I. The original mating numbers are used, which give essentially a chronologic sequence, but in analysis we shall disregard sequence, in favor of a more logical approach. Matings were made in individual cages unless otherwise noted, and embryonic development was observed at regular intervals by candling. Eggs showing dying or dead embryos or no development were opened for better examination. Many eggs or squabs were given to foster parents to be raised. Records were made of all events. Each bird's identification number was stamped on an aluminum clinch band which was then placed on the bird's leg. Repeated checks have eliminated error, so far as we are aware. At intervals most of the hybrids were carefully described. Photographs, some in color, supplement many descriptions. At death the skins, or wings and tails, were preserved for future comparisons. Such comparisons were carefully made for this study, but the whole collection has since been destroyed.

Artificial insemination techniques have been developed by Owen (1941) for pigeons and doves, and he has successfully applied them in aid of this study (see mating E455 data).

The pigeons, doves, and hybrids have received essentially the same care. Food consisted of wheat, cracked corn, kafir, and Canada field peas, mixed with about 1 per cent. crude cod-liver oil. A mineral supplement of crushed oyster shell was afforded at all times, and every week coarse sand mixed with salt (about 10 per cent.) and dampened was given. Occasionally other supplements such as charcoal or ground green stuffs were given, but their value was dubious. Water was almost continuously available to the birds, in covered cups, and occasionally in bath pans.

General Characterization of the Hybrids

The F_1 hybrids (males) were sterile or nearly so, in spite of general virility and eagerness to mate. In backcrosses to pigeons, only two eggs showed any development out of 770 incubated. In backcrosses to ring doves, results were better (Table II); over 60 eggs were fertile in 1727 incubated, and 14 hatched. Suchetet (1896) and Whitman (1919) also had some success with this backcross.

TABLE II

Summaries of Breeding Results in Backcrosses of Hybrids

HYBRID mated with	MATINGS	TOTAL EGGS	BROKEN, LOST, etc.	INFERTILE	DEAD EMBRYOS FIRST WEEK	DEAD EMBRYOS LATER	HATCHED	% FERTILITY
♀ PIGEON	12	452	2	448	2	0	0	< .5
	*	320	0 ?	320 ?	0	0	0	0
♀ RING DOVE	36	1588	85	1464	20	8	11	< 2.5
	9**	231	7	?	20	?	3	10.0

* A pen of hybrid males with female pigeons, matings not individually recorded but approximately 10.

** Matings E387, E399, E635, E664, E686, E738, E807, and E920; fertility not recorded in detail.

The surviving backcross offspring were all males; although these too were sexually active, further backcrossing to the ring dove gave only one possibly developing egg in over 660 incubated (Table III). This result was disappointing and quite unexpected.

Suchetet (1896) reported normal sperms in his hybrids, while Guyer (1900) and Smith (1912) found abnormal spermatogenesis and mostly abnormal—often double-sized—sperms. Unpublished observations on sperms of some of our hybrids have been made by Dr. T. S. Painter, and these are presented in Table IV. The hybrids differed markedly among themselves, but in general there were many abnormal sperms. Many germ cells apparently break down at

TABLE III

SUMMARY OF RESULTS FROM MATINGS OF ¾ RING DOVE – ¼ PIGEON MALES WITH FEMALE RING DOVES

♂ #	NUMBER of MATINGS	TOTAL EGGS	BROKEN, LOST, etc.	INFERTILE	DEAD EMBRYOS	
					EARLY	LATER
D196U	2	20	1	19	0	0
D234J_3	6	251	4	247	0	0
D234J_4	5	151	4	147	0	0
D262E_2	4	209	9	199	1 (?)	0
E387T	2	46	0	46	0	0
E635B	1	?	1	3	0	0
TOTAL	20	681	19	661	1 (?)	0

the first meiotic division (Painter and Cole, 1943). No cytological or sperm study has been made of the backcross individuals.

Unfortunately no truly wild specimens of either parental species have been used in this cross. The nearest approximation to a "type hybrid" was first described by de Montlezun (1905); the cross was said to be a wild-type common pigeon female with the ordinary or "blond" ring dove male. The following is a direct translation:

> Dorsal: in general, plumage deep pearly gray from the base of the beak to the end of the tail coverts; scapulars and large coverts of the wing lightly washed with russet; primary remiges dark brownish gray with blackish shafts, and marked at the tips and the edges of the outer barbs with a whitish border. Rectrices gray of a slightly lighter shade than the remiges; the three laterals terminating in a white band about a fifth of their length; the two [outer] laterals bordered outwardly with white; the middle part of the neck marked with a collar arranged like that of doves, but not very obvious except when the feathers are raised.
>
> Ventral: throat whitish gray to neck, then purplish gray to the sternum; breast, abdomen, and sub-caudals pearly gray, with a very light shade near the anus; ventral wing coverts gray, almost white; ventral aspect of

TABLE IV

NOTES ON BIOPSY MATERIAL FROM TESTES OF SEVERAL HYBRIDS AGED 1 TO 3 YEARS (T. S. PAINTER, 1928, UNPUBL.), WITH BREEDING-TEST DATA WHERE AVAILABLE

HYBRID ♂	SPERMATOZOA		FERTILITY
	NORMAL	ABNORMAL	
D48J5	----	many	0 in 32 eggs
D69C	rare	"	1 dead embryo in 91 eggs
D69D	fair number	"	4 in 144 eggs (2 hatch)
D108V	----	few (testes not active)	1 late dead embryo in 54
D108W	a few	many	2 dead embryos in 24 eggs
D108E2	many	"	0 in 67 eggs
D108S3	"	----	
D110U	"	----	
D110W	rare	many	0 in 38 eggs
D110U2	many	----	
D110E3	rare	fair number	6 in 136 eggs (2 hatch)
D147D	----	----	0 in 30 eggs
D147E	----	many *	0 in 42 eggs
D148N	----	----	1 dead embryo in 29 eggs
D148E2	rare	fair number *	0 in 30 eggs
D148T2	----	" " *	0 in 33 eggs
D148W2	rare	" " *	
D148X2	"	" " *	
D148Z2	----	few *	
D148E3	----	----	
D165H	a few	many	

----= none found. *= highly abnormal.

remiges of lighter shade than dorsal; rectrices blackish with white bands at the sides very apparent; flanks and thighs pearly gray. Feet violet rosy; claws and beak black horn color; iris red orange. The coo of the hybrids resembles that of both the pigeon and the dove.

Measurements:

total length	330 mm.	wing tip length	200 mm.
wing span	580 mm.	tail length	120 mm.
wing length	260 mm.	center toe with claw	33 mm.

beak length from forehead 35 mm. [*sic*; = 15 mm.?]

While this description is fairly meticulous and conforms to convention, it lacks parental data for comparison. We

have prepared something more serviceable, from some of our data (Table V). Plumage coloration and pattern are not included but will be dealt with again in connection with pattern inheritance in crosses with different breeds.

TABLE V

COMPARISON OF DIFFERING FEATURES (EXCEPT COLORATION OF PLUMAGE) IN TYPICAL PIGEONS, RING DOVES, AND THEIR MALE HYBRIDS

	PIGEONS		RING DOVES		HYBRIDS
FEATURE	♀ (1)	♂ (1)	♀ (Av. of 8)	♂ (Av. of 8)	♂ (Av. of 7)
Body weight (gm.)	322	397	140	158	225
Tarsus Length (mm.)	29	30	21	21	27
Middle Toe " "	28	30	23	25	27
Beak Tip to Center of Eye (mm.)	32	34.5	29.5	30	33
Middle Rectrix (mm.)	129	146	121	132	135
Primary Remex Third outer (mm.)	178	205	138	146	161
Nostril Cere	chalky		not chalky		intermediate
Tibiotarsal (hock) joint	feathered anteriorly		bare		bare
Neck Feathers	iridescent, no "collar"		no iridescence, black "collar" with white edge		broad scaly collar patch, tips of these feathers iridescent
"Greeting laugh"	none		5 - 6 notes		2 - 3 notes
Courting Coo	rolling-staccato		rippling, melodious		undulating, hoarse
Pugnacity	usually marked		usually slight		usually marked

Tarsus was measured from posterior angle of hock to anterior angle with toe; toe measurement was from base of claw to angle with tarsus. Feather lengths are entire (plucked). The pigeons measured here are Archangels, the male being 2664J, and the hybrids are all sons of this male (mating E21).

To the casual observer, the hybrid appears roughly intermediate between the parents. A much more detailed statistical study is probably needed, but some curious relations

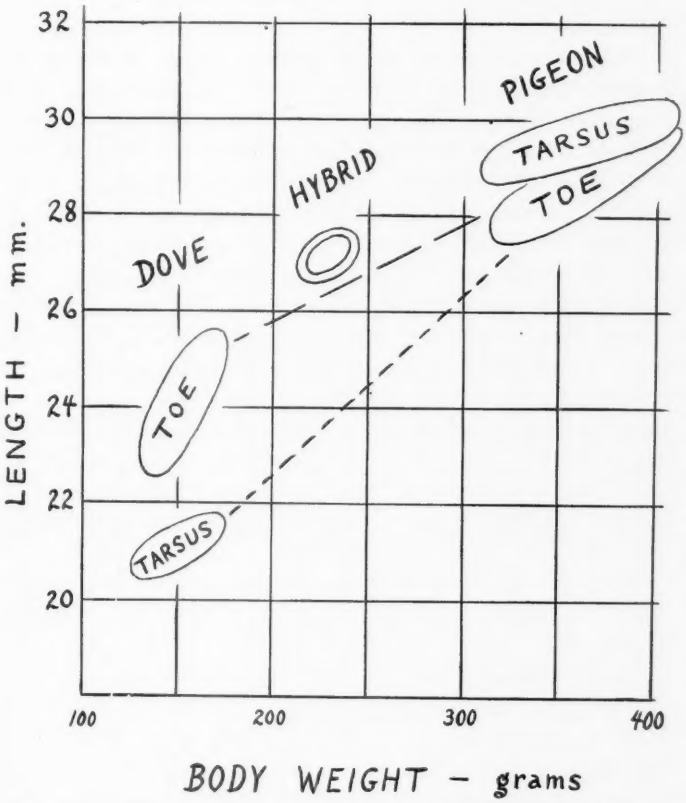

FIG. 1. A graphic comparison of doves and pigeons of both sexes and their male hybrids, as to body weight, tarsus length, and middle toe length. Broken lines between the male values for pigeons and doves indicate that the hybrid has a relatively long tarsus. See also Table IV.

are found in the table. These become more apparent in graphic form (Fig. 1). The variation of middle toe length shows a marked "sex influence," and the hybrid's length is about at the point expected in proportion to body weight for males. On the other hand, tarsus length in the parents seems to have a linear relation to body weight, regardless of sex, and the hybrid's tarsus is markedly longer than

would be predicted on the basis of proportion. In fact, the hybrid might be said to show "heterosis" for this feature.

The ratio of rectrix:remex feather lengths is markedly different between the parental species; in the pigeon it is about 0.7, while in the ring dove it is about 0.9. In the hybrids the ratio is about 0.84, somewhat more like the dove than intermediate. Plotting the ratios against body weight, however, gives an almost rectilinear series. Though possibly fortuitous, these relationships are most interesting. Further studies of this sort have not been pursued.

The backcross adult males (¾ dove, ¼ pigeon) were intermediate in size and form between the F_1 and the ring dove. D196U was rather delicate and weak-legged, but the others were robust and sexually active. Like the F_1 these birds had a pugnacious disposition and a hoarse undulating coo, but the neck collar was much more like that of the dove. Unfortunately no measurements were preserved of these interesting birds. Photographs of two are given in Plate 7.

Since the egg of the ring dove is hardly half as heavy as that of a pigeon, one might reason that hybrids developing in the dove egg would be at a disadvantage. Actually, hybrids hatching from pigeon eggs are initially almost twice the weight of those from dove eggs. Dr. Ray D. Owen has made a comparison of growth rates of some hybrids from the reciprocal crosses, and his data are presented in Fig. 2. Two conclusions may be drawn from them: first, that the initial disadvantage of the hybrids from dove eggs is largely overcome in the early weeks of growth; second, the differences between the females of the reciprocal crosses probably do not involve sex-linkage of size inheritance.

Another question relating to size of eggs concerns the incubation periods of hybrids from reciprocal crosses. These have been recorded and tabulated in detail, but it is difficult to draw definite conclusions because of the great variability. Such factors as degree of constancy of parental hovering, and nutritional state of the mother can not be accounted for properly. The mean incubation period for doves is

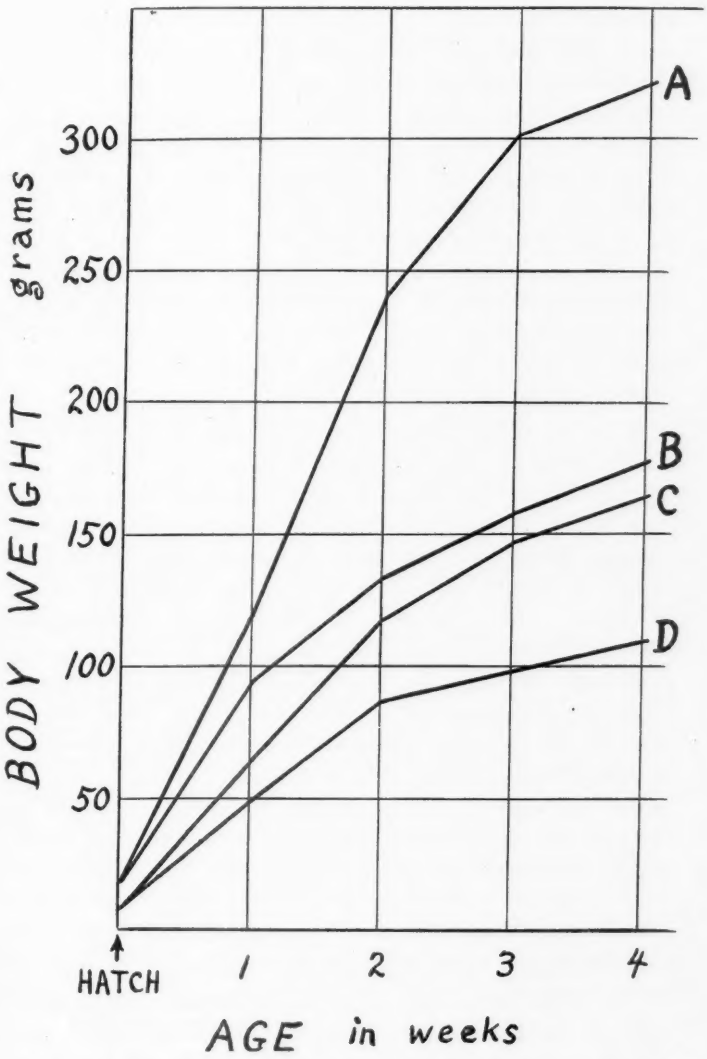

FIG. 2. Growth curves, based on data of Dr. Ray D. Owen. A: pigeons (21 averaged); B: female hybrids from male dove by female pigeon (2 averaged); C: male hybrids from male pigeon by female dove (11 averaged); and D: ring doves (26 averaged).

about 14 days, while that of pigeons is about 17 days. Apparently the limits of ordinary deviation are about ± 1 day, though hatching can be retarded longer by cooling the eggs intermittently in the last stages of incubation.

The hybrids have incubation periods ranging mostly from 14 to 18 days (inclusive); for over 200 hybrids in dove eggs, the average was about 15 to 16 days. For seven hybrids in pigeon eggs the variation was from 15 to 19 days, the average being about 16 days. Since five of the latter seven hybrids were females, there is some lack of comparability, but no definite sex difference in incubation periods is discernible. We may tentatively conclude that the hybrids rank between the parental types in incubation periods, regardless of the direction of the cross.

As noted above, female hybrids from male pigeon by female dove are rare, delicate, and short-lived. The two reaching maturity here were $D29D_3$, which lived six months, and E522A, which lived nearly 16 months. Even though mature, these birds had apparently juvenile ovaries at death. By contrast, four of the females obtained from the reciprocal cross (mating E455, by artificial insemination) reached maturity and lived from three to seven years, a practically normal span for the parental types. Moreover, these females appeared moderately robust; they showed some sexual behavior, and their ovaries became practically mature, though no eggs were ever laid.

The life spans of the male hybrids seem to be fully as long as those of the parental types. The maximum age of pigeons is not known, but few survive 15 years. Whitman (1919, vol. II, p. 130) gives 12 years as the age of one hybrid, and several of ours have lived over 10 years.

The backcross offspring have also been fairly long-lived. D196U lived nearly two and one-half years; $D234J_3$ lived over 14 years; $D234J_4$ lived seven and one-half years; and $D262E_2$ also lived seven and one-half years.

A study of the immunogenetic features in this cross was reported by Irwin and Cole (1936). The erythrocyte anti-

gens of the hybrids include those of both parental species, though some variation was noted in the pigeon antigens represented. Also there was a new antigenic property or "hybrid substance," which was interpreted as an effect of complementary action of genes governing antigenic properties. (Analogous effects have been noted in other species hybrids.) No female hybrids were used in the immunogenetic studies, but three backcross males were tested: $D234J_3$, $D234J_4$, and $D262E_2$. The last two were quite similar to the F_1 in antigenic properties, while $D234J_3$ was more like the dove, having few pigeon antigens and little or no hybrid substance.

Riddle (1947) studied the basal metabolic rate of pigeons, ring doves, and their hybrids (his Table 41, and page 196). In general the doves had the highest rate and the hybrids were intermediate. The findings may simply relate to differences in the ratio of surface area to body weight.

Inheritance of Mutant Traits

We shall assume for simplicity that the various domestic types of pigeons and doves have been derived from their respective wild species types exclusively by mutation and selection. Hence any trait differing from the wild-type condition will be termed a "mutant."

About 20 mutant traits have been involved in our hybridizations. For the majority of the traits the inheritance is known to be simple, although in some combinations there are characteristic interactions or epistasis. The latter phenomena are particularly common with mutants involving coloration. For illustrations and reviews on the genetics of these traits the reader is referred to Levi (1941).

A list of the traits is presented in Table VI. Two have been omitted: "tamability" and "tumbling"; these have been practically impossible to classify under our conditions and have been largely ignored. However, it may be stated that we have found the hybrids about as tame as the parental types.

TABLE VI

MUTANT TRAITS USED

MUTANT TRAIT	SOURCE	GENES	SEX-linked	MATINGS
blond (plumage color)	Dove	1 rec.	yes	D770, E455, etc.
"white" (" ")	"	1 rec. allele of blond	yes	D69, D108, D128, D165, D210, E564.
dilute (" ")	Pigeon	1 rec.	yes	D4, D5, D29, D48, D69, D93, D110, D118, D128, D165, D210, E21, E437, E511, E522, E564, E992.
brown (" ")	"	1 rec.	yes	D311.
ash-red (" ")	"	1 dom. allele of brown	yes	D5, D110, D118, D152, D234, D311, E507.
almond (" ")	"	1 dom.	yes	G235.
rec. red (" ")	"	1 rec.	No	D48.
bronze (" ")	"	1? part. dom.	No	D183, E21.
grizzle (" ")	"	1 dom.	No	D147, D148, D199, D770.
piebald (white areas)	"	1? dom. and alleles and modifiers	No	D5, D107, D108, D118, D123, D130, D251, E321, E455, E485, E496, E507
spread (plumage color pattern)	"	1 dom.	No	D48, D107, D108, D110, D130, D152, D251, D311.
checker (" ")	"	1 dom.	No	E21, E455.
"T" pattern (" ")	"	1 dom. allele of checker	No	D5, D29, D69, D118, E321, E496, E507.
smoky (" " ")	"	1 rec.	No	E21.
pearl (white iris pigment)	"	1 rec.	No	D4, D29, D48, D69, D108, D128, D130, D165, D210.
crest (occipital whorl)	"	1 rec.	No	E21, E507.
frill (neck and crop whorls)	"	1? rec.?	No?	D311, E437, E507, E511, E522, E564, E992.
silky (plumage structure)	"	1 dom.	No	E321, E496.
foot feathering	"	1 or 2 part. dom.	No	D4, D48, D93, D128, D165, D210.
short beak	"	2? part. dom.	No	D108, D311, E437, E507, E511, E522, E564, E992
grease-quills	"	1? dom.	No	E21.

Rec. = recessive; dom. = dominant.

It seems best to anticipate the details of the results with the summarizing statement that the expression of the mutant traits in the hybrids could be fairly well predicted from previous knowledge of the genetics of the parental species. Thus, the autosomal recessive traits crest, frill, pearl, smoky, and recessive red were recessive in the hybrid. Since all these mutants are from the pigeons, we may conclude that the ring dove contributed normal dominant alleles of each. Similarly, the dominant or partially dominant mutants have all given recognizable phenotypes in the hybrid. However, these require some special comment.

Whitman (1919) in his discourse on wing-color patterns and orthogenetic evolution in the Columbidae did not consider the present cross; the phenotypes are certainly not in harmony with his theory. It seems best at this point to consider again the "type hybrid." The ring dove has no pattern on the wing, while typical *Columba livia* has two black bars across the wings. The hybrid, as described by de Montlezun (1905; see above), closely approximates the dove in pattern. In our series, pigeon male 1710H and others were of the wild-type bar pattern, and their hybrid offspring also seem very much like the dove in pattern (Plate 1). One might conclude that the dove condition is simply dominant. The pattern mutants, however, complicate the situation.

Earlier studies by Hollander (1938) have shown that there are at least three mutant pattern alleles in the pigeon: barless, with plain wings like the dove's (but not used in our hybridizations); checker; and "T" pattern, in which the wings are mostly black (or other dense pigmentation). Barless is recessive to the wild-type bar, checker is dominant to both of these, and "T" is dominant over all the above. The barless pattern of the dove then can hardly be identical genetically with that of the pigeon, since in the hybrid the barless condition seems to dominate.

A comparison of "type hybrids" with hybrids from checker or "T"-pattern pigeons has shown that the pat-

terns are not absent but merely *obscured*. It is not very difficult to distinguish them in the hybrids, despite the superficial similarity to the ring dove's patternless wing (Plates 1 and 5). This is a previously unrecognized and rather surprising fact; we may conclude, therefore, that the ring dove lacks both the checker and "T"-pattern factors, and that it has a dominant wing-pattern inhibitor or suppressor.

The color pattern of the tail feathers is also of considerable interest. The parental species differ markedly in this feature. In the pigeon the end of the tail has a broad transverse band of black, with a faint and narrow lighter terminal edge. The ring dove on the other hand has a very broad terminal whitish zone. The hybrids resemble the ring dove decidedly in this respect (see plates).

It was pointed out in an earlier paragraph that the tail feathers of the ring dove are relatively much longer than those of the pigeon, and that the hybrid is closer to the ring dove than to the pigeon in this particular. Now it seems desirable to point out the correlation between relative length and the development of the terminal whitish zone. It seems worth considering the possibility that this part of the tail is extremely abbreviated in the pigeon, and more or less consequentially the whitish zone fails to develop in that species. The rest of the tail pattern would, on this hypothesis, be essentially homologous (including the black band).

The "spread" mutant of the pigeon is a pattern-suppressing gene; its presence in an otherwise wild-type pigeon produces a fairly uniform black pigmentation. Ring-dove hybrids receiving this gene are similarly affected, except for the terminal zone of the tail, which still develops a considerable whitening.

Piebaldness of several sorts has been involved in our crosses. Practically every hybrid from the Baldhead Tumblers showed some white feathers in the same areas as the pigeon parent (Plate 2). On the other hand, hybrids from

the Turbit showed only a small white patch on the rump (Plates 5 and 6). The remaining matings involved mongrels heterozygous for piebaldness; some of the hybrids from them had small areas of white.

White pigeons are usually "extreme piebald," genetically. Whitman (1919, vol. 2, page 129), citing Günther, noted that hybrids from a cross of white Fantail by ring dove were bluish in color, but he does not specifically state that there were no white feathers. Whitman made the same cross himself (pages 130–131 and Table 111); one offspring is adequately described, and it had some white feathers on the head and elsewhere. Smith (1912) crossed the Magpie pigeon with the ring dove and obtained three hybrids, which he states were uniformly pigmented except for the whitish tail band. The possibility of a few white feathers was not definitely ruled out, however. In summary, the hybrids generally show about as much white as would be expected in comparable crosses of piebald with non-piebald breeds of pigeons.

All the matings involving the mutants for "silky" plumage and for foot feathering showed segregation since the pigeons were heterozygous. "Silky" was perfectly obvious in the hybrids, but foot feathers seemed somewhat reduced in size and number as compared with the pigeon parent. The presence of the mutant condition was indisputable in some hybrids, however.

Short beak, characteristic of Tumblers, Owls, and Turbits used in these crosses, is partially dominant to normal length, but may not be unifactorial. The hybrids from these pigeons have had somewhat shorter beaks than other hybrids, just as in pigeon breed crosses.

"Grease quills" are modified fluff feathers along the dorso-lateral curve of the body between wing and tail. They are waxy and unopened, in contrast to the normal rich fluffiness. This mutant trait is dominant or partially dominant (Cole and Hollander, unpublished). It is found in several breeds, *e.g.*, the Archangel, and is associated with

a gloss or semi-iridescent sheen over the other plumage. All the Archangel hybrids (E21) have shown the trait.

We have not employed the Fantail breed in our hybridizations. The supernumerary tail feathers of this breed depend on a multifactorial inheritance, but there is known to be some dominance. Whitman records a cross of a male Fantail having 19 rectrices with a ring dove (12 rectrices) which produced two offspring, one having 13, the other 14 rectrices (1919, vol. 2, pages 130, 131, and Table 111). The Fantail breed seems to have been fairly successful in hybridization, but only these two detailed descriptions of the hybrids were preserved by Whitman. He also notes that from a Tumbler with 13 rectrices he obtained one hybrid with the same number (Table 114).

Sex-linked traits pose some problems of their own; outstanding is the fact that all the ring doves employed in our crosses have had either the "blond" or the "white" mutant colorations, which form a multiple allelic series with the dark wild type (Cole, 1930).

It was early discovered that if a "dilute" pigeon were used with either blond or white dove, the hybrids were all blond (= dilute), quite distinct from the slaty "type hybrids." Apparently therefore blond and dilute are essentially the same genetically.

Both "ash-red" and "almond," mutants of the pigeon, are conspicuous types of light colorations with dark flecks (usually) scattered in the plumage. In almost all cases the pigeon parents having these mutants were heterozygous. The hybrids receiving the traits were remarkably similar to the pigeons in coloration, even to the flecking.

One male pigeon (see mating 311) was heterozygous for ash-red and its recessive mutant allele "brown" or "chocolate." Seven hybrid offspring were recorded in plumage and all were males. Of the seven, four were black and three ash-red. The four blacks must have received the brown mutant from the father and a normal allele (black pigmentation) from the ring dove. If sex reversal can occur in

pigeon–dove hybrids, as suggested by Riddle (1925), there is no genetic evidence for it here; no brown (genetic female) offspring was produced.

The use of sex-linked mutants for proof of sex of hybrids is also possible in other matings. Where the sire is a dove and the dam a pigeon, all the hybrids we have obtained show agreement in genetic and anatomical sex. Mating D770 produced two described offspring, one blond and one slaty. The blond was female, the other male, as expected. Mating E455 produced four blond offspring, all females, as expected. Still another bit of evidence is obtainable with the ash-red mutant. The dark flecks with this coloration are ordinarily of black pigment in males but brown in females. The ash-red hybrids have shown only blackish flecks. Here again genetic maleness is indicated.

The inheritance of the mutant traits in the backcrosses is difficult to follow because of scarcity. It seems best to comment on the specimens individually.

D196U was from a black Baldhead Tumbler hybrid. Three traits might be expected to segregate visibly: "spread," piebaldness, and blond. But apparently there was no segregation; D196U was fairly blackish, with a couple of white tail feathers.

D197A died at about two weeks of age, but resembled a blond ring dove. The parentage was supposedly about the same as D196U, but the fact that D197A was from the first egg of the mating, as well as its similarity to a pure ring dove, might cast some doubt on the paternity.

$D234J_3$ and $D234J_4$ were practically identical in appearance. Their hybrid sire was dilute spread ash-red (with dilute flecks), and the two backcross males also appeared to have these mutants. However, determination of the presence of spread is not easy in this combination.

$D262C_2$ died at about three weeks of age and was described as "blond." $D262E_2$ was also a "blond." The hybrid sire of these birds had only the dilute mutant from the pigeon.

E387T, E635B, and E920C_2 were all "blond." Other mutants (checker, grease quills) were not noted, but may have been present. The hybrid sires of all these three were dilute.

Discussion

Of initial and perhaps of deepest interest are the problems of genetic incompatibility in this cross. (1) Why should practically all female hybrids die as embryos in matings of pigeon males with doves, while in the reciprocal cross female hybrids survive readily? (2) Why should the proportions of normal-appearing and abnormal sperms be so strikingly different among the hybrids? (3) Why should backcrosses of male hybrids with pigeons give no fertility while with ring doves they give over 2 per cent. fertility? (4) What genetic (or other) basis can account for the degree of fertility in the latter backcross? (5) Why should the backcross specimens be only males and totally sterile?

Since Painter and Cole (1943) have already dealt with problem (1) fairly thoroughly, we shall only add that egg size seems to have little significance here. If the pigeon egg were more favorable for hybrid development than the dove egg, we should expect the reverse situation regarding fertility in problem (3). The problem is by no means cleared up, however, either in this cross or others. Reciprocal cross differences are not uncommon, either in regard to sex ratio or to fertility. Sandnes and Landauer (1938) report that the cross of pheasant by chicken gives a marked excess of males in one direction but not in the reciprocal. Owen (1941b) noted that fertility in crosses of guinea fowl by chicken is always low and that no embryos survived long from guinea fowl sires. The female duck hybrids from reciprocal intergeneric crosses, reported by Crew and Koller (1936), are widely different in many respects. These authors note, however, that the incubation period of all the hybrids is practically the same as that of the Muscovy parent—a week longer than that of the other parent. In

mammals some similar situations have been found, though in these the male hybrid is more likely to be sterile than the female (Craft, 1938).

Studies of spermatogenesis in the pigeon–dove hybrids are in agreement that there are disturbances, but there is disagreement on detail. Smith (1912) states that synapsis fails, so that irregular masses of chromatin are separated; following this aberrant first (reduction) division, he found no second division, but direct development of spermatids and consequent formation of (often) double-sized sperms. By contrast, Painter and Cole (1943) state that synapsis is intimate, though breakdown may often follow. Probably all the authorities are correct in part, the hybrids being exceedingly variable in this feature. At present we can only surmise why this variability should exist; perhaps breed differences which seem insignificant, otherwise, may take on more fundamental importance when balanced against chromosomes of another genus. If intimate synapsis usually occurs, we must conclude that genic rather than chromosomal differences are of major importance here.

At least, sperms are ordinarily produced by these hybrids, even though the sperms are often abnormal. A different condition was found by Crew and Koller (1936) in their study of sterility of intergeneric duck hybrids; the testes showed great irregularities of meiosis and complete failure of spermiogenesis.

Since most of the pigeon by dove hybrids produce plenty of sperms, of which some seem normal, one still can be puzzled over the contrasting results of the backcrosses. We can think of no reasonable answer to problem (3) as yet, except some peculiar sort of incompatibility.

The problems (4) and (5) might be resolved by assuming that most of the surviving "¾ dove, ¼ pigeon" specimens are actually triploid—⅔ dove, ⅓ pigeon. This assumption is not ruled out by any of the few facts that we know so far. The frequency of F_1 double-size (essentially diploid) sperms is high; no females would be expected to result, though

intersexes might occur; total sterility would be quite within expectations; and " weakening " of the phenotypes of dominant genes from the pigeon might occur—as seems likely in D196U and D234. One would also expect to find no segregation of heterozygous genes in the hybrids in formation of diploid sperms; presumably the backcross offspring of any one hybrid should be practically identical. Actually, no segregation seems to have occurred in the production of D196U; since three genes were heterozygous in his sire, the result is impressive. However, the only other informative mating is D234. The two offspring were phenotypically identical, and showed no segregation. However, immunogenetically they differed somewhat. Here is a suggestion that triploidy may not be the explanation, though no better seems available. But even if triploidy is involved in this backcross, why should it not occur equally readily in the reciprocal backcross?

The expression of mutants in the F_1 needs little discussion. Our findings agree very well with those of Serebrovsky (1929) in pheasant–chicken hybrids, namely, that recessive mutants act as recessives and dominants act as dominants, with little modification. A more distant example is found in the canary–finch hybrids, which show piebaldness if the canary parent contributes that mutant (Duncker, 1928, page 125).

What information we have points conclusively against sex reversal in the hybrids, *i.e.*, genetic males or females as determined by sex-linked mutant traits are also anatomically of that sex.

It is hoped that this study, though unsatisfactory in many respects, will serve to orient and perhaps stimulate further investigation.

Summary

This is a general study of problems in the hybridization of the domestic pigeon with the domestic ring dove, and includes both original data and a review of pertinent literature.

Pigeons of small size have given best results in natural cross matings, but artificial insemination is possible. Female hybrids rarely result from pigeon sires, but are readily produced in the reciprocal cross. Surviving female hybrids have produced no eggs. Male hybrids produced abundant sperms but varied greatly in the proportion of sperms which seem normal. In backcrosses to pigeons practically no fertility was shown, but in backcrosses to doves over 2 per cent. fertility has resulted, and nine eggs hatched. All backcross specimens were males and have proved sterile but testes and semen have not been examined.

In regard to size and form the " type hybrid " is roughly intermediate between the parental species. More detailed study reveals complications as to proportions. For one feature, tarsus length, the proportions seem to differ from both parental types.

A study of color-patterns suggests that the ring dove possesses dominant suppressor factors which inhibit the expression of wing patterns inherited from the pigeon. Tail color patterns are complicated by differences in proportionate feather lengths in the species; the hybrid is strongly influenced by the ring-dove inheritance. It is suggested that the terminal zone of the pigeon tail feather is rudimentary as compared with that of the ring dove.

The expression of some 20 mutant genes in the hybrids has been studied. With minor exceptions, these factors behaved no differently than in the pure species: recessives were not expressed in the hybrids, while most of the dominants gave their customary phenotypes. Sex-linked mutants are transmitted as expected in each sex. Results with the pigeon color mutant " brown " afford direct evidence that sex reversal does not occur. Essential identity is found between the pigeon mutant " dilute " and the dove mutant " blond."

It is suggested that the backcross specimens may owe their peculiarities to triploidy, though evidence is as yet inadequate.

ACKNOWLEDGMENT

The protracted nature of this investigation has been made possible by a series of assistants without whose routine aid the records and observational details would be much less extensive and complete. Special thanks are due to Dr. Ray D. Owen and Dr. T. S. Painter for their research data. Dr. M. R. Irwin has been very helpful in completing the manuscript since the death of Dr. Cole.

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PLATE 1. Above: pigeon ♂ 1710H, showing "dilute" plumage coloration, feathered tarsi and toes, and "pearl" iris color. Note that the wing flight feathers extend nearly to the end of the tail. Above left: ♀ 1808A "blond" ring dove. Lower left: hybrid ♂ D4H from above parents; wings spread to show faint indication of bar pattern. Lower right: hybrid ♂ D69C, side view; plumage color "light blond" (dilute/white), with denser pattern ("T" pattern).

PLATE 2. Above: white ring dove ♀. Center: black Baldhead Tumbler pigeon ♂ 2140A. Below: hybrid ♂ D108F_2, son of above; "spread" pattern and piebaldness from pigeon parent are evident.

PLATE 3. Above: grizzle Tippler pigeon ♂ 2121A. Center: grizzle hybrid ♂ D147E, son of above. Below: head and neck of hybrid ♂ D69C (see also Plate 1).

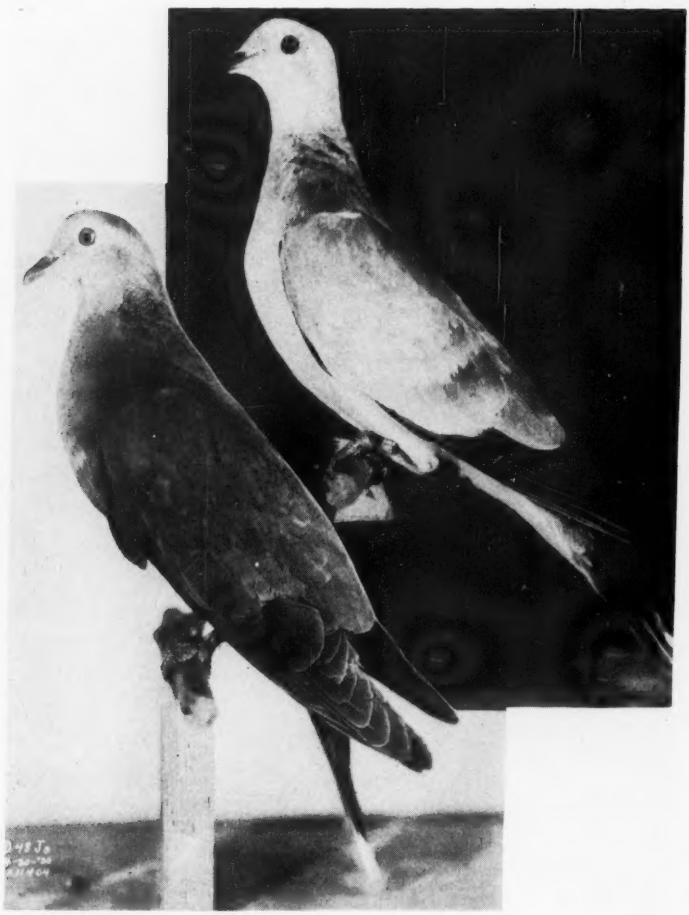

PLATE 4. Above: grizzle hybrid ♂ D148X$_4$. Below: "dun" hybrid ♂ D48J$_5$. The latter shows dilute/blond coloration, with "spread" pattern; note also feathered tarsus.

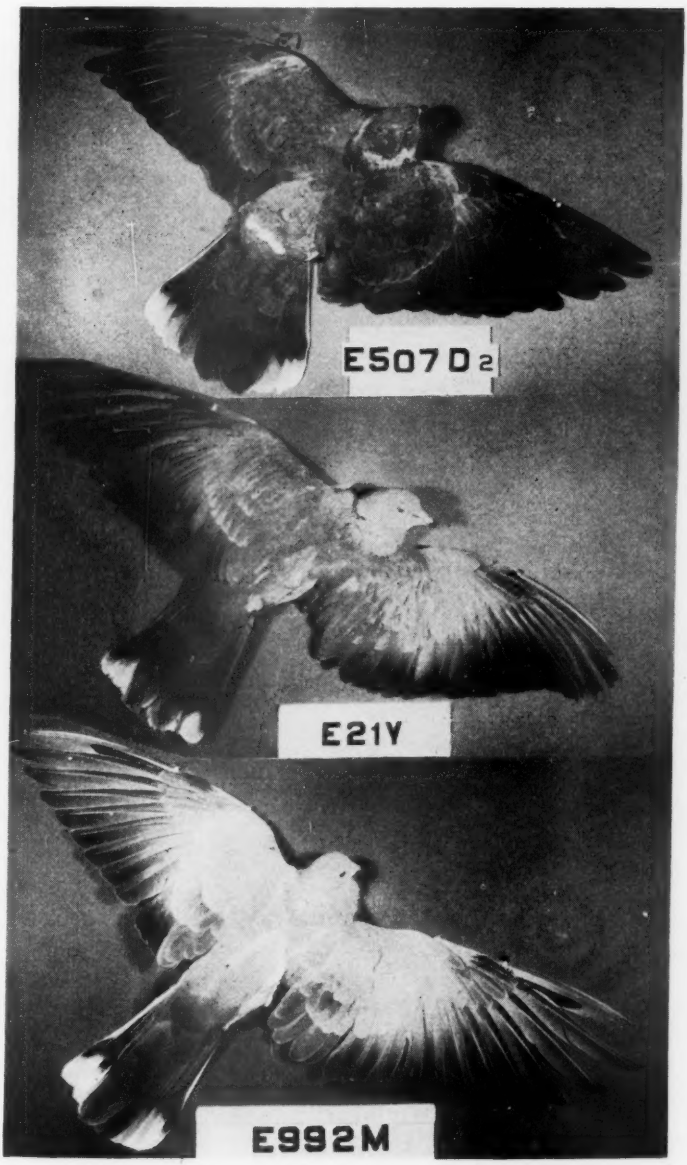

PLATE 5. Hybrid ♂ E507D$_2$ shows the "T" pattern; hybrid ♂ E21Y shows the "checker" pattern; and hybrid ♂ E992M shows faint indication of the bar pattern. The latter two hybrids are dilute/blond.

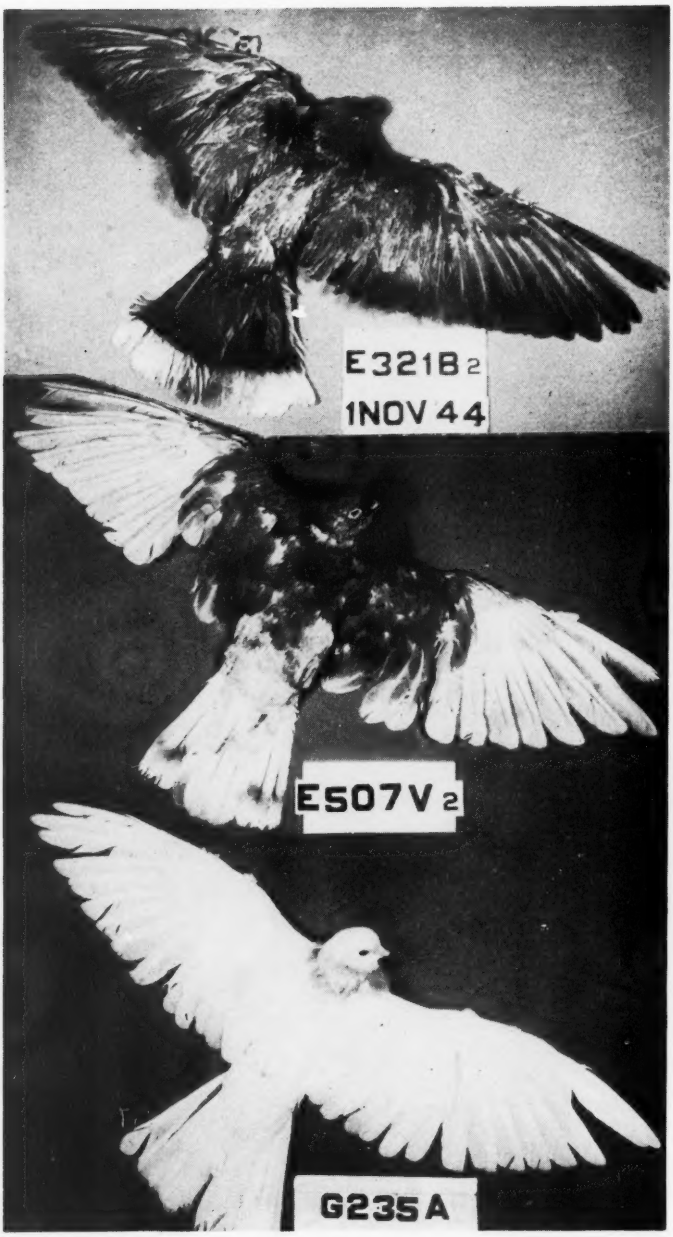

PLATE 6. Hybrid ♂ E321B$_2$ has "silky" plumage and "spread" pattern (less definite in the tail). Hybrid ♂ E507V$_2$ shows the "ash-red" color, with "T" pattern. Hybrid ♂ G235A shows "almond" coloration.

PLATE 7. Dorsal view of backcross hybrid D196U, showing spread pattern and one white tail feather. Side view of backcross hybrid D262E$_2$, resembling blond ring dove considerably.

NOTES ON SOME DECAPOD CRUSTACEANS FROM THE VIRGIN ISLANDS

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DURING the summer of 1949 the author had occasion, while visiting his friend, Svend Mylner, Esq., on St. Thomas, to make a collection of littoral and amphibious decapods. While all the 13 species encountered are known from the Caribbean area (Rathbun, 1933, and Schmitt, 1935) two of them, the small hermit-crab, *Calcinus tibicen* (Herbst), and the mangrove-crab, *Goniopsis cruentata* (Latreille), have not been recorded before from the Virgin Islands. The following notes include a list of the species with some remarks on nomenclature and a few observations made in the field.

The following species were collected:

MACRURA

Panulirus argus Latreille

ANOMURA

Calcinus tibicen (Herbst)
Coenobita diogenes (Catesby) = *C. clypeatus* (Herbst)

BRACHYURA

Mithrax forceps (A. Milne Edwards)
Callinectes marginatus (A. Milne Edwards)
Chloridiella longimana (H. Milne Edwards)
Ocypoda arenaria (Catesby) = *O. quadrata* Fabricius
Uca rapax (Smith)
Grapsus maculatus (Catesby) = *G. grapsus* Linné
Goniopsis cruentata (Latreille)
Pachygrapsus transversus (Gibbes)
Sesarma ricordi (H. Milne Edwards)
Cardisoma terrestris (Catesby) = *C. guanhumi* Latreille

The author has deliberately given preference to the names given four of these species by Marc Catesby, who first found them on the Bahama Islands and described and depicted

them in his admirable and beautiful book, "Natural History of Carolina, Florida and the Bahama Islands." The importance of this work warranted its publication in English and French in three editions, in 1734, 1755 and 1773, and in a Latin and German translation in 1750. To invalidate the names Catesby gave his new species because he happened to use perfectly good Latin binomial nomenclature a generation before Linné published the 10th edition of his "Systema Naturae" in 1758 may be in accord with the *Code,* but in the humble opinion of the author no majority vote should be permitted to deprive a scientist of full credit for his accomplishments on the sole ground that he did his good work too early. The arbitrary selection of the year 1758 as the Year 1 of zoological nomenclature appears to the author unwarranted from a historical viewpoint and not required under the circumstances. The "Linnéan system of nomenclature" was no invention of Linné.

The following field observations were noted at the time of collection.

Panulirus argus, the local "lobster," was found in considerable numbers, particularly among the mangrove roots on isolated mangrove islets in Mangrove Lagoon near the southeastern extremity of St. Thomas. A specimen demonstrated the tremendous power of its tail muscles. In trying to evade capture, it jumped from under water several feet up in the air, landing on a breakwater.

Calcinus tibicen, a small littoral hermit-crab, was found abundantly on the coral reef flats along the shores of St. Thomas and of Water Island.

Coenobita diogenes, the land-hermit-crab, was common from the shore about Mangrove Lagoon up to the top of the highest hills of St. Thomas. One specimen was encountered on the island of St. John at an altitude of over 1000 feet. A female carrying eggs was taken on St. John several hundred yards from the seashore.

Callinectes marginatus was plentiful on all sand flats and

coral reef flats. Most of the specimens were young and rather small.

Chloridiella longimana was abundant among the branches of coral colonies, especially on Water Island. They were collected in numbers merely by breaking blocks of coral on the surface of a flat rock.

Ocypoda arenaria, the sand-crab, was found in a group of some 20 burrows on a small sandy beach on the south side of Mangrove Lagoon. The holes were located at a distance of 10 to 20 yards from the water's edge. When the locality was visited in the early afternoon, all the crabs were down in their burrows and had to be dug out by hand.

Uca rapax, perhaps a Caribbean variety of the northern fiddler-crab, *U. pugnax,* was found on the north side of Mangrove Lagoon. They were present in very large numbers around their burrows in the dark-colored mud between the mangrove roots at the water's edge.

Grapsus maculatus, the red mottled rock-crab, is represented in the collection by only a single specimen, a female carrying eggs, caught in the water among stones along the rocky shore immediately west of St. Thomas harbor.

Goniopsis cruentata, the mangrove-crab, was encountered while walking along the sandy beach on the south side of Mangrove Lagoon. Upon approaching a small water-filled hole some 10 yards from the shore, a considerable number of these colorful crabs rushed out of the water and up into the bushes on the landward side of the pool. It was with considerable difficulty that a colored boy was able to catch a few for the collection.

Cardisoma terrestris, the land-crab, was found in two large colonies, the one a few hundred yards from the sea, in the open woods along the road to Magens Bay, the other among the trees around a well along the road to Mangrove Lagoon near Mariendal. During the day most of the crabs sit at the entrance to their burrows venturing only rarely a short distance away. They are impossible to catch by day but are easily taken by night using a flashlight. Boys

make money from catching and selling them for food. Most of the crabs are yellowish brown in color; while some specimens are almost black, and others are a bright red or blue. Some of the crabs are found with almost smooth walking legs, but the majority have dark strong spines on these appendages. One specimen in the collection is unusual in that the two claws are of equal size.

Of the three remaining species, *Mithrax forceps, Pachygrapsus transversus* and *Sesarma recordi,* only single specimens were obtained.

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